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**Glyphosate Tolerance in Cotton: Challenges of Weed Resistance  
and Pathways to Sustainable Management**

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**Abstract:** Roundup Ready Flex (RRF) cotton has brand new essentially recreated the modern-day system of weed control, using glyphosate during an entire growing season. This rate of variability facilitates use of conservation tillage approximation, simplifies the functioning of the farms, and has been accredited with better results of soil health findings, all of which help in bringing about the sustainability of cotton production. However, its reliance on glyphosate has led to the development of glyphosate resistant strains of weeds, which jeopardizes the sustainability of the system. Perturbations of the ecological sturdiness are fanned due to declines in agricultural assortment and changing lines of weed species in unity. Integrated Weed Management (IWM) has thus emerged as a suitable solution and proposed the application of coordinated chemical, cultural, mechanical, and biological control strategies to alleviate the resistance and protect sustainable productivity. This current discourse hence outlines the advantages and constraints of RRF cotton, as well as explains the need to use adaptive, integrated policies to ensure the sustainability of cotton weed management in the future. It highlights the contribution of precision agriculture, trait stacking and an appropriate policy framework in sustaining cotton agroecosystems at the same level of productivity that is also environmentally safe.

**Keywords:** Roundup Ready Flex, Glyphosate, Chemical, Integrated Weed Management, Strategies, Ecological, Precision Agriculture and Agroecosystems.

## **1. Introduction**

Cotton (*Gossypium hirsutum* L.) is the most widely cultivated natural fiber crop and plays a crucial role in the global agricultural economy. The cotton manufacturing industry has traditionally utilized the fibers themselves, but when cotton is produced, two important by-products cottonseed oil and cottonseed meal are produced, which in turn are used in large volumetric doses as

stimulants in the animal-feed and human-food industries respectively. Cotton production nurses the income needs of millions of residents who live on natural environments, especially the arid and semi-arid ecological regions (Zhang *et al.*, 2023). The last decades have shown an increasing rate of exploitation of this species over the globe in the context of improving high-technology fiber production, more precise agronomic management practices, and the commercialization of genetically engineered varieties (Singh *et al.*, 2021).

Weed infestation is one of the most tedious and unending proofs that are causing havoc in the cotton production. The aggressive competition of vital resources, such as water, light, and nutrients that the weed pressure exposes, leads to yield losses of up to 60 % on unmanaged plots (Ali *et al.*, 2022). Moreover, the presence of weeds compromises fiber quality, affects harvesting of the plants, as well as increases insect pest and pathogen harboring and the costs of production (Martins *et al.*, 2020). Conventional weed-management processes manual weeding, mechanical tillage operation, and application of broad-spectrum herbicides are time consuming, costly and environmentally unfriendly especially in large scale management (Kumar *et al.*, 2023).

It was the commercialization of the herbicide tolerant (HT) crops that brought a paradigmatic shift in managing weeds. This was a milestone brought about by the availability of RR cotton in 1997, that is, strains that are engineered to be able to endure glyphosate a broad-spectrum, non-selective herbicide that is characterized by its efficiency and cost effectiveness which is made available by the introduction of the cp4-EPSPS gene. RR cotton, however, was modified to provide resistance only to early glyphosate sprays, but application later in the growing stage often caused phytotoxicity, especially in reproductive parts, hence, making the yield and quality poor (Huang *et al.*, 2023).

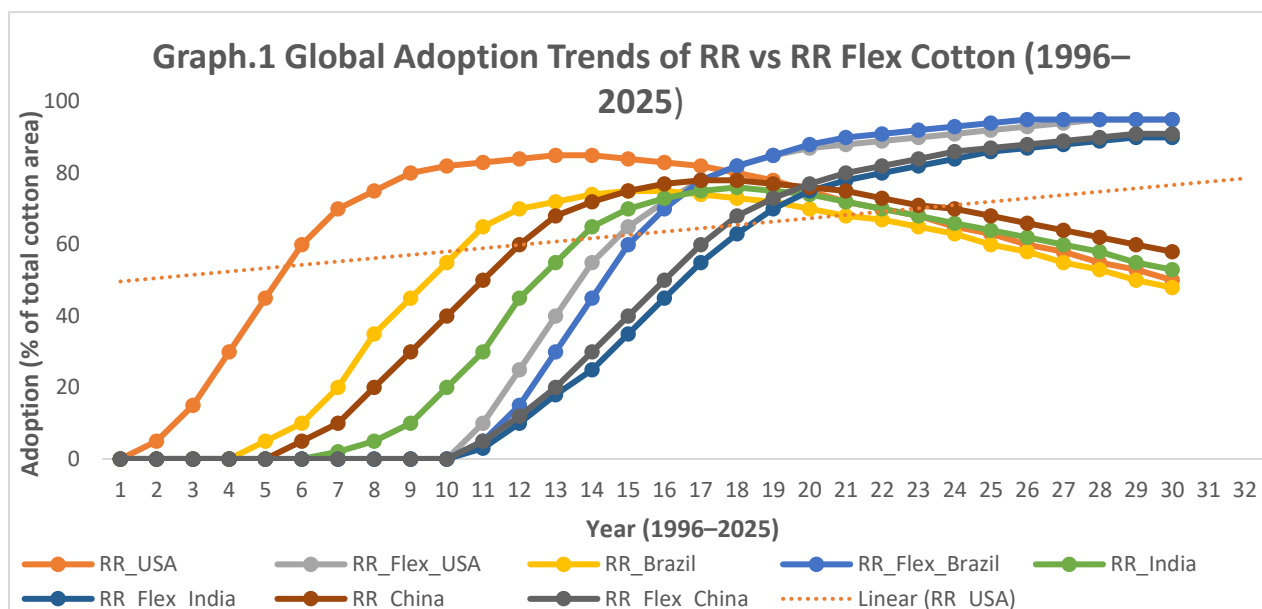
RRF cotton (an improved version introduced in 2006 of RR) was introduced, it was popularly expected that it would help to overcome some constraints that previously existed in other HT cultivars. Unlike earlier HT products, RRF cotton has an even, tissue-wide advent of the cp4-EPSPS transgene, as well as in reproductive organs and, therefore, allows glyphosate to be applied at any point in the growth cycle without affecting the crop integrity (Nguyen *et al.*, 2021). This modification of genes broadens the boundaries of weed control, lowers or eliminates tilling, and advances medicinal agricultural aims via the practice of soil health (Moraes *et al.*, 2022).

With expanding and unremitting usage of the compound glyphosate, there has been a shocking increase in the populations of glyphosate-resistant (GR) weeds. Of the over 50 weed species that have developed glyphosate resistance in diverse agro-ecological environments since the commercial introduction of the herbicide-tolerant cotton, some of the most problematic ones are *Amaranthus palmeri*, *Ambrosia trifida*, *Conyza canadensis* and *Lolium rigidum*. The increasing rates of GR weeds imminently degrade the long-term effectiveness of glyphosate by threatening to shift the stringent management of weeds in the fields that require urgently, IWRM (Carvalho *et al.*, 2024).

Successful IWM would include herbicide rotation with different modes of action, the application of cover crops, the use of mechanical weed control whenever possible, and diversified cropping to break the continual evolution preferences of weeds (Dias *et al.*, 2023). These approaches are not only necessary to maintain the productivity benefits that are brought by HT cotton technologies especially RRF, but also to delay the development of the resistance, preserve biodiversity and achieve sustainability in cotton production systems (Pereira *et al.*, 2023).

The current review critically looks into the historical process of RRF cotton, agronomic consequences, and limiting factors of RRF cotton, reviews the evolution, and worldwide distribution of GR weeds, and evaluates the capacity of integrated resistance management strategies to deal with those problems in existing and future cropping systems.

Roundup Ready cotton was first commercialized in 1997, revolutionizing weed management through glyphosate tolerance. Later, Roundup Ready Flex cotton, introduced in 2006, provided extended over the top glyphosate application tolerance. The adoption patterns of these technologies across major cotton producing countries are shown in Graph.1 (Mahoney et al.,



2022).

Herbicide tolerant cotton varieties have transformed weed management in cotton cultivation. The 1<sup>st</sup> generation RR trait allowed glyphosate use only during early plant development, which provided effective but time limited control. The improved RRF trait broadened this tolerance across the entire growing season, permitting multiple applications with greater safety and efficiency. Table 1 summarizes the differences between these two technologies in terms of tolerance, advantages, and constraints.

**Table 1. Glyphosate resistance traits in Roundup Ready vs Roundup Ready Flex cotton**

Trait	Herbicide Tolerance	Benefits	Limitations	Citations
Roundup Ready (RR)	Tolerates glyphosate only in early growth stages (up to 4-5 leaf stage, before flowering)	Effective early season weed control, reduced need for tillage	Limited spray window; crop injury if sprayed later;	Nguyen et al. (2021)

			higher risk of weed escapes	
Roundup Ready Flex (RRF)	Extended tolerance throughout the growing season, including flowering and boll development	Flexible weed control timing; multiple applications allowed; reduced crop injury; improved yield potential	Dependence on glyphosate increases risk of resistance evolution in weeds	Moreno and Javed. (2024)

## 2. Roundup Ready and Roundup Ready Flex Cotton

RR cotton, which was commercialized in the year 1997 was a paradigm-shifting development in agro-biotechnology. With genetic engineering, it was infused with the EPS-SPS gene of *Agrobacterium* sp. strain CP4 which has made it glyphosate tolerant without causing a direct attack to the crops. The weed-seeding option imparted by the HT phenomenon was a simplified resource in controlling farming weeds and together with the manual classifications essential as it significantly reduced the dependence on hand pulling and tilling practices (Singh *et al.*, 2022).

RR cotton was rapidly implemented in all the top cotton producers when it was released. By the year 2003, about seventy percent of all cotton acreage in the United States was planted with RR varieties, which were driven by better user convenience, reduced cost of production, and no-till farming systems eligibility (Fernandez *et al.*, 2021). However, initial RR hybrids could only be sprayed with glyphosate when plants were in the 4-6 leaf phenology and when the treatment was applied during the flowering and boll setting periods, it was often a cause of boll abscission and reduced reproduction (Lopez *et al.*, 2023).

The evolution in biotechnology in the agricultural sector has been fast and since 2006 there has been RRF cotton. This cultivar also comprises of improved regulatory sequences, which has enabled the use of glyphosate in all parts of the crop cycle, even in the highly sensitive reproductive phases (Nguyen *et al.*, 2024). The accompanying gain in user flexibility and weed control capability, on an all-year-round basis, makes RRF cotton a key tool of choice in maximizing weed control-management. Repeated, season-long applications of glyphosate can manage late-emerging population of weed species and agricultural weed seed bank, thus enhancing the yield potential

and long-time soil health (Castro *et al.*, 2022). In addition, the glyphosate resistance profile of RRF cotton enables the use of conservation agriculture, enhances soil structure, and reduces erosion through a reduction in the amount of ploughing (Valdez *et al.*, 2023).

The large and ongoing usage of the glyphosate-based herbicides in glyphosate-resistant RR and glyphosate-resistant plus various other resistance traits stacks-cotton systems has imposed considerable selective pressure on the biota of the weeds. This pressure has increased the rate of evolution of glyphosate resistant weed populations therefore presenting a great danger to the sustainability of glyphosate tolerant cotton culture in the long term. As a result, methods of IWM and planned herbicide rotation systems have become critical towards avoiding development of resistance (Martinez *et al.*, 2025).

### **3. Mechanism of Tolerance**

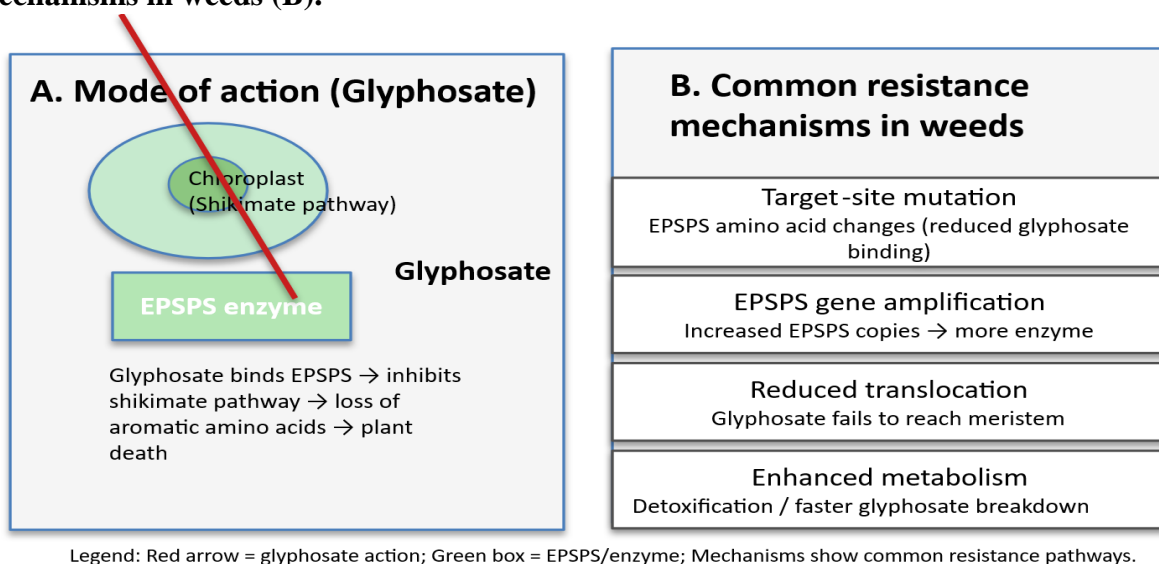
Mechanistic base of glyphosate tolerance in RR and RRF cotton is based under principles of biochemical engineering in plants whereby emphasis is paid to shikimate pathway. Glyphosate is a non-selective, systemic herbicide which acts as an inhibitor of the enzyme 5-enolpyruvylshikimic acid, 3-phosphate synthase/ ester or (EPSPS) which is essential in the production of aromatic amino acids phenylalanine, tyrosine and tryptophan. In traditional plants, its metabolic pathway breakdown leads to the accumulation of shikimate and metabolic imbalances, thus the eventual death of the plant (Rehman *et al.*, 2021). In order to counter this susceptibility, RR and RRF cotton genetically modified varieties have been modified to produce an alteration of the EPSPS enzyme found in the *Agrobacterium* strain CP4. The cp4-EPSPS is a gene that encodes the EPSPS variant (glyphosate resistance protein) that does not alter the catalytic activity and, as a result, enables the plant to continue producing amino acids despite the administration of the herbicide (Ali *et al.*, 2023).

The issue in cotton breeding in the recent past has been reduced crop competitiveness and rise of pest resistance that has prompted more attention being paid to transgenic approaches which increase resistance to pests in non-target tissues and developmental stages. CaMV 35S promoter has in the past granted global but time limited promotion of genes, but super regulatory factor promoters exhibited an enhanced performance since they can offer long lasting expression in varieties of tissues and developmental stages. The constitutive promoter systems provide an excellent level of resistance to environmental stress and disruption of reproduction, which makes them essential to the performance of crops (Iqbal *et al.*, 2022).

The successor to RR, RRF cotton is a repeating process that uses newer architectures on its promoters that allow gene expression throughout the life of the plant. Long expression window is a factor that gives the plant tolerance to several applications or high doses of the herbicide without damaging the crop, thus, providing the growers with more flexibility in terms of approach to managing the weeds (Moreno *et al.* 2024). Moreover, partial-season tolerance of gene expression in both apical meristem and fruit produces must undergo strategic or partial localization to yield positive performances in stable yields when glyphosate is applied persistently over a long period. By discovering that herbicide-specific patterns of expression, dose response, and tissue-specific expression all positively influence tolerance to herbicide regimes and lead to optimization of the plant architecture and agronomic parameter ultimately stabilizing cotton yield (Yaseen *et al.*, 2023).

Glyphosate inhibits the EPSPS enzyme in the shikimate pathway, blocking the synthesis of aromatic amino acids required for plant growth. Over time, certain weed species have developed resistance through mechanisms such as target-site mutations, EPSPS gene amplification, altered translocation, and enhanced metabolism. These adaptations are illustrated in Figure 1.

**Figure 1. Glyphosate action on the EPSPS pathway (A) and evolved resistance mechanisms in weeds (B).**



## 4. Weed Management in Cotton

### 4.1 Pre-Herbicide Techniques

Before the development of chemical herbicides, the only methods by which weeds could be managed in cotton production were in large part manual, mechanical, and cultural. The most common physical intervention methods were hand weeding, hoeing, and inter-row cultivation, which have the characteristic features of intense labor demand and extended periods (Ahmed *et al.*, 2021). The effectiveness of these methods was subject to the taxonomy of the weed, agro-climatic conditions, and managements in place since partial suppression of the weeds is achievable in these methods (Kumar *et al.*, 2020). Mechanical intervention, especially the routine one, encouraged soil compaction and erosion that followed after repetitive tillage, hence reducing the fertility level and potential longevity of crops (Singh *et al.*, 2022). This applies to such agronomic cultural controls like planting density manipulation, crop rotation and mulching, although they have been found to restrict weed emergence, it has not been effective in fighting intense infestations in the areas of high weed-pressure (Nawaz *et al.*, 2023).

The agronomic practices that were used in the mid-twentieth century ended up enhancing soil disturbance and hence encouraging the germination of the weed seeds held in the seed bank. The traditional farm methods though had their shortcomings still provided a foundation upon which the current model of IWM has been built where the pre mm would be sanitation, crop competitiveness and prompt action (Bibi *et al.*, 2024). Practically, growers implemented preventive measures that included timely irrigation, clean seedbed and stale seedbed manipulation as a way of preventing weed emergence or delay. However, the rising labor cost, as well as the necessity to increase crop productivity, made these practices economically unviable in the long-term perspective (Shah *et al.*, 2023). The later invention of synthetic herbicides revolutionized modern day weed control in agriculture because they provided a steady and effective inhibition especially in large cotton land areas. Systems based on the use of herbicides allowed intervening in time, selectively and cheaply, eventually through better crop performance and an economical reduction in labor demand (Tanveer *et al.*, 2025).

#### **4.2 Systems Based on Glyphosate**

Commercialization of glyphosate resistant (RR) and glyphosate resistant-flex (RRF) cotton became a ground-breaking product in weed management and helped in Glyphosate application even after the product has emerged, without affecting productivity of the crop in any negative way. This development simplified weed management operations, minimized tillage, lowered labor needs, and helped in carrying out any conservation activity (Norsworthy *et al.*, 2021). Still, a long-

term and sole reliance on glyphosate has given rise to glyphosate-resistant weed populations, especially that of *Amaranthus tuberculatus* and *Lolium rigidus*, which undermines the effectiveness of a glyphosate-based approach in the long term (Vink *et al.*, 2022).

The wide-ranging effect of glyphosate means that it exerts a significant selection pressure to accelerate the development and evolution of resistance as well as endangering sustainable management of weeds (Chandi *et al.*, 2020). In order to solve these problems, herbicide rotation, cover cropping, and mechanical weed management forms of integrated methods are essential to reduce the build-up of resistance and extend the glyphosate lifespan (Bunithan *et al.*, 2023).

## **5. Weed Resistance to Glyphosate**

### **5.1 Emergence of Glyphosate-Resistant Weeds**

The high and prolonged use of glyphosate has led to the emergence of weed populations that are resistant to herbicides very fast particularly when used in cotton cropping system. More than fifty of the weed taxa including *Amaranthus palmeri*, *Ambrosia trifida*, *Conyza canadensis* and *Lolium rigidus* had developed glyphosate resistance (Gaines *et al.*, 2020). These taxa cause significant difficulties to crop production and they become an economical factor of weed control increasing the prices of using herbicides and consequently raising the labor supply needs.

Resistance has developed because of improper long-term reliance on glyphosate as the sole method of using the product in weed control strategies. Single herbicide application over time could prove to put a lot of pressure on selection pressure and therefore ultimately leading to resistant biotypes of weeds to prevail. In the long-term, such populations grow and become more difficult to manage, which makes glyphosate-based systems ineffective and unsustainable. Nor should they allow resistance to keep spreading and an IWM practice is therefore necessary. They include application of rotating herbicides with varied mechanisms of acting, mechanical cultivation, warrants of applying cover crops and prompt weed observation. These tactics combine to diminish the degree of selection force, but also enhance prolonged year to year weed controls and sustainability of cotton production (Norsworthy *et al.*, 2021).

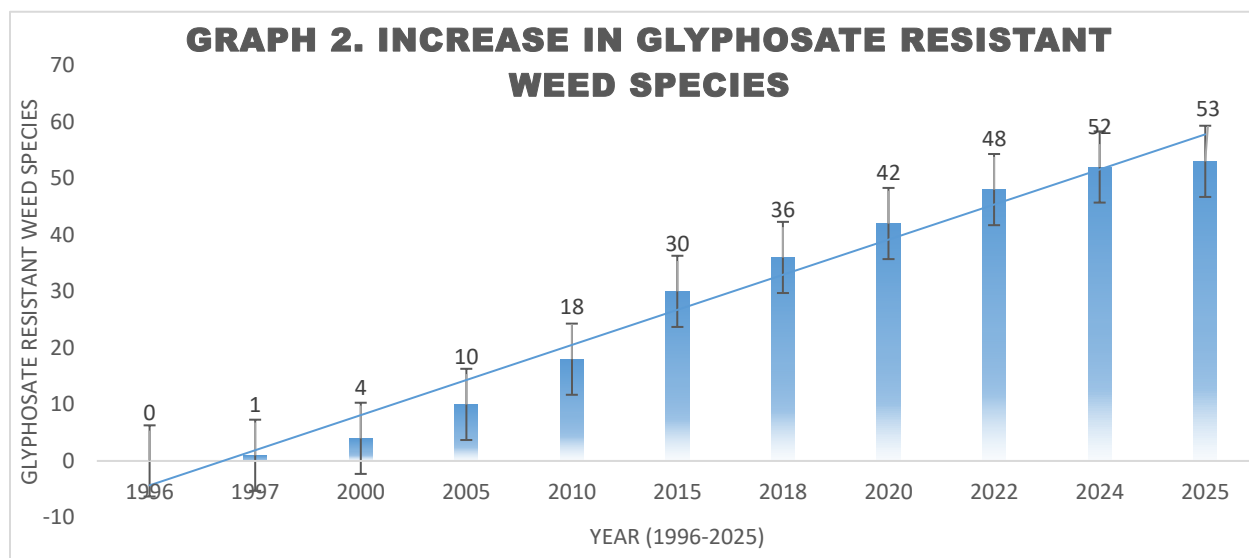
### **5.2 Anti-Resistance Mechanisms**

The problem of resistance in weeds to the use of glyphosate has developed several adaptive systems that would jeopardize the sustainability of Roundup Ready Flex-cotton systems in the long term. Target-site resistance is one feature identified where mutation in the *EPSPS* (5-enolpyruvylshikimate-3-phosphate synthase) gene takes place. The mutations change the binding

site, and, as a result, the enzyme is no longer sensitive to glyphosate but becomes less inhibited by it (Gaines *et al.*, 2020). The *EPSPS* gene amplification is another significant resistance tactic as resistant species of weeds end up copying countless copies of the *EPSPS* gene. This surplus causes a surge in the amount of EPSPS enzyme thus the plant does not malfunction badly with soddy normal metabolic process with the presence of glyphosate (Dill, 2022). This has been heavily documented in troublesome species such as the *Amaranthus palmeri* and presents very significant challenge to measures to control through chemicals.

Besides, non-target site resistance has been developed. Certain weeds who are resistant have the ability of metabolizing glyphosate in a much better metabolism and converting it to harmless levels. The other group has an altered translocation of glyphosate, in which movement of the herbicide within the plant is minimized, thus unable to reach the meristem that is the most vulnerable to the herbicide. The multilateral resistance pathways point toward the dire necessity of cross-discipline and diversified weed management strategies (Heap *et a.*, 2024).

The expansion of glyphosate tolerant cotton cultivation has coincided with a notable rise in glyphosate-resistant weed species across the globe. This pattern demonstrates the ecological risks of relying too heavily on a single herbicide mode of action and emphasizes the importance of integrating diverse management strategies. Graph 2 depicts the steady increase in resistant weed cases over the last three decades, representing a significant challenge to the long-term sustainability of glyphosate-based production systems.



## 6. Strategies for Weed Resistance Management

## **6.1 Herbicide Rotation**

The development of glyphosate-resistant weeds is the primary reason that requires the implementation of herbicide rotation techniques as one of the most important resistance management approaches. Continuous and exclusive use of glyphosate renders great selection pressure on weed populations that favors the survival of resistant ones. A switching herbicide that are used with contrasting modes of action reduces such selection and decreases the probability of resistance achieving. Herbicide rotation depends on rotating the active ingredients, seasons or within one growing pattern with the aim of subjecting the weeds to different biochemical routes. As an illustration, mixing/intercropping of glyphosate with other herbicide e.g. glufosinate, dicamba, or 2,4-D can extend spectrum of weed management and prevent the occurrence of resistant biotypes. Such multi-mechanism approaches will diminish the adaptive capacity of weeds and extend the lifespan of current herbicide technologies (Norsworthy *et al.*, 2023).

More than chemical rotation, it is ideal that farmers will alternate crops and customize weed management strategies according to local weeds flora and history two of herbicides. Crop rotation alters the time of the appearance of the weeds, their strength and their spectrum, conditioning a biased selection of a particular herbicide resistance factor. Rotations involving crops and herbicide rotation have also proved to be highly effective in extended delaying the occurrence of resistance and subsequent long-term herbicide lifespan (Neve *et al.*, 2020).

## **6.2 Herbicides with Residual Activity**

Residual herbicides have come to constitute a rife means of controlling glyphosate-resistant weed populations within cotton-based cropping systems. The characteristic feature of residual herbicides is that they are active in the soil to a long period of time after application in order to prevent seed germination of weeds or early growth of weeds. This prolonged action is more crucial in early part of cotton development, whereby weed competition may severely delay the establishment of crop, make stands uneven and finally submit the yield potential to decrease. These herbicides give a preemptive strike and lessen the number of post-emergence sprays and increase management windows (Jha *et al.*, 2021).

Major strength of including residual herbicides is that they reduce the selective pressure on glyphosate and therefore reduce rate of resistance development and possible spread. Herbicides, in combination through different modes of action, herbicides with soil residual properties will expand the weed management program since glyphosate resistant biotypes have been more

frequently observed because of repeated applications of the same mode of action. In order to widen the scope of weed control, if necessary, it is possible to use products that contain some form of active ingredients such as metolachlor, flumioxazin, pyroxasulfone or diuron and apply alone or together within glyphosate. Not only does this method ensure an even longer control period into the middle of the season, but it also avoids the phenomenon known as weed escapes, where the weeds can grow and seed on their own and repopulate the soil seed bank, making another season even more difficult to manage (Dille *et al.*, 2022).

Additionally, incorporation of residual herbicides also leads to a more sustainable and tougher weed management system due to better season-on-season control and less frequent post application of interventions. When used with other agriculture practices (like crop rotation practices and tillage, cover crops and herbicide mode-of-action rotation practice), residual herbicides will reduce the possibility of resistance development and add resilience to the cotton production system as a whole. An aggressive resistance management plan that involves herbicide layering and residual effectiveness aids in conservation of the performance of glyphosate and other herbicides in the long run. The use of residual herbicides in a diversified integrated weed management (IWM) system is critical in slowing down the production of herbicide resistant populations of weeds and securing sustainable crop production (Neve *et al.*, 2020).

### **Mechanical and Cultural Controls**

Establishment of IWM is anchored precisely on a synergetic combination of mechanical and cultural strategies aimed at mitigating the glyphosate addiction and enhancing resistance of cropping systems. Of the cultural approaches, crop rotation holds a central point, as it is the only measure that can break the synchrony between specific weed populations by rotating crops that differ significantly in both planting time and canopy structure, and in their needs of resources (Neve *et al.*, 2020). This temporal and spatial variant limits the ability of the weeds to adapt and minimizes the persistence of effective weeds over the production cycle. Simultaneously with the crop rotation, there is increased population density created by narrow inter-row spacings and even more intensified intrados densities through the cultivation of very competitive cultivars, which increases the intra-field competition of the crop in terms of capturing the light, water, and nutrients. All of these practices hinder the growth survival consequences of weeds and encourage the crop vigor in its initial periods, and at the same time, this strategy dissipates the prevalence and persistence of glyphosate-resistant biotypes (Singh, *et al.*, 2022).

The other cultural practice worth noting is cover crops which are becoming an incorporated part of cotton systems in enhancing the aspect of weed suppression. Other cover crops e.g. cereal rye, hairy vetch or clover emit allelopathic chemicals that have the effect of preventing reproduction of weed seeds through germination and/or early seedling development, and also render ground surfaces physically impenetrable to weeds due to the growth of vegetation (Dille *et al.*, 2022).

Cover crops are considered an efficacious tool to control weeds due to their dual effect, the physical and the chemical one. Further, after termination, the cover crops can leave behind their biomass that can be used as mulch and it can still control the weeds during the growing season. Cover crops minimize input costs and increase ecological sustainability through the reduced use of chemical herbicides since planting reduces the number of weeds that develop during the early seasons of the year. Tillage is also an effective method of weeds management, which can be used by mechanically controlling glyphosate-resistant weeds, especially pre-planting (Mahoney *et al.*, 2022).

With pre-plant tillage, weeds can be physically uprooted before they grow, weed seeds can be covered to a greater depth than the optimal germination level, and the weed seed bank can be disturbed. Although the purpose of conservation tillage systems is to reduce disturbance of the soil, tactical tillage operations on essential intervals, when completed in the right time and with well thought-out planning, can considerably diminish that population of weeds that are troublesome without jeopardizing the success of the soil over the long-term. Also, integration of mechanical practices together with cultural tactics boosts the diversity and health of the biodiversity, and improves soil structure along with microbial activity which makes the cropping system more resilient and productive. Such combinations are required to establish sustainable weed control strategies that are long-term and minimize resistance development and promote agroecosystem fitness (Mahoney *et al.*, 2022).

### **6.3 Integrated Weed Management (IWM)**

IWM entails a sustainable and comprehensive system that uses a heterogeneous approach to weed population control by means of a multiplicity of control strategies, such as chemical, mechanical, cultural, and biological procedures to ensure population control as well as minimum occurrence of resistance to herbicides. The growing problem of emergent glyphosate-resistant weeds, especially in cotton systems where the glyphosate-tolerant cultivars are being broadly

applied, has taught that there is the need to shift away from one control measure (Norsworthy *et al.*, 2021).

Numerous synergistic combinations of weed management approaches found in IWM aim at immunization against selection pressure by variability in selection pressure and postponement of evolution of weed biotypes resistant to herbicides. The chemical operating principle of IWM is the rotation of the mode-of-action-groups of herbicides, tank-mates and pre-emergence (residual) herbicides. It has been found that by combining herbicides, like glufosinate, dicamba, or 2,4-D with glyphosate, it is possible to enhance weed spectrum management and risk of resistance (Evans *et al.*, 2025).

Some cultural practices too have proved to inhibit the growth of weeds through methods like crop rotation, cover cropping and narrowing row gaps all these methods help disturb the life cycle of the weeds and make crop more competitive. Another aspect used in weed suppression is the use of allelopathic cover crops which is also achieved through physical cover which is the cover crop, and its chemical inhibition on the germination of the weeds (Mirsky *et al.*, 2023).

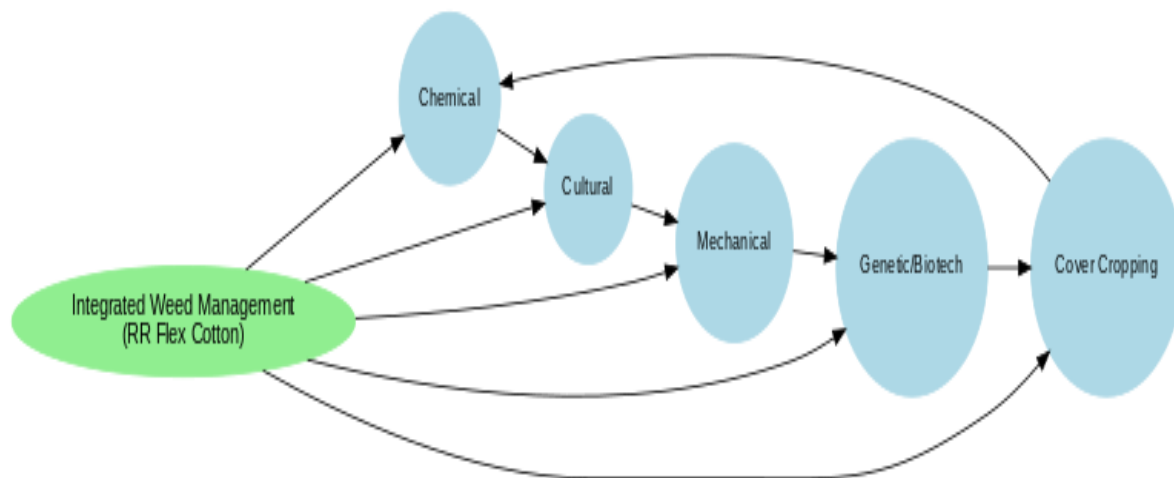
Use of mechanical methods such as deep tillage, inter and row tiling and stale seedbed preparations are done to bring down the seedbank and kill emerging seedlings before plants can establish. Although these are labor-intensive procedures they are of great help where there is integrated system whereby there is a reduced usage of herbicides. The harvest weed seed control (HWSC) has proved to be useful in controlling the weed seeds during harvest in many areas and preventing their recapture into the soil seedbank (Walsh *et al.*, 2022). Biological control not as advanced in the row crop operations but holds long-term sustainable potential in the form of intro- or appreciation of such natural enemies as pathogens, insects or competitive plant species, which help suppress the population of weeds. In cotton, biocontrol has not yet been embraced in a significant way; nevertheless, promising investigations are being done to ensure that biocontrol is viable in relation to some difficult weeds (Shaner *et al.*, 2020).

The extremely important aspect of IWM is the active field surveillance and management of the control measures. This involves scouting to identify the weed escapes, mapping the occurrence of resistant population and altering their practices according to the weed biology and performance of herbicides as well as field history (Heap *et al.*, 2024). IWM is not a formula but a changing and rather location-based mechanism that has to be refreshed frequently and must be as efficient as possible. The bottom line is that IWM is a success whose ability to enhance

sustainability, minimize impact to the environment, and maintain herbicide performance in future is a plus to its success. It allows cotton farmers to operate with a high level of productivity and safeguard the health of agroecosystems, biodiversity in soils as well as the economics of the service. The use of IWM principles by every member of the agricultural industry needs to be coordinated to confront the advancing menace of herbicide-tolerant weeds (Peterson *et al.*, 2023).

Sustainable use of RR Flex cotton requires more than dependence on glyphosate alone. An IWM strategy that brings together chemical, cultural, mechanical, and genetic measures is essential. Figure 2 illustrates this cycle, showing how these approaches complement one another to limit resistance development and improve overall weed control.

**Figure 2. Integrated weed management strategies in Roundup Ready Flex cotton**



### **Advantages and Limitations of RRF Cotton**

RRF cotton produces a wide range of agronomic, environmental and economic benefits to the cotton growers, hence, the preferred technology by the current cotton grower technologies. Among the most significant advantages is the increased controllability of weeds because of the broader application period of the glyphosate herbicide, which means that farmers have been able to be more flexible and efficient in their control of weeds along the crop cycle (Brookes *et al.*, 2020). This leads to a better crop stand and yield performance, with much competition hindering crops at their early stages of growth (Harker *et al.*, 2021). RRF cotton also allows glyphosate to be applied over the top at any stage after the 4-leaf stage resulting in an improved means of suppressing earlier and late-emerging weeds.

Besides the above, RRF cotton has one greater advantage which is that it enhances cost-effectiveness and minimization of labor. Since it is just a single mode of action, glyphosate removes the need to use complicated tank mixes of herbicides/ extensive hand/machine weeding and hence, farmers can save on herbicides and machines/equipment and manual labor in their farms (Gianessi *et al.*, 2022). It also benefits in terms of the reduction of field trips and cutting of the cost of production, since the simplified protocol of weed management will neither demand so many field trips nor cause problems related to fuel expenditure and abrasion of machinery (Norsworthy *et al.*, 2022). RRF cotton is environmentally friendly, to the level that it promotes the conservation tillage such that the structure of the soils is retained in addition to soils being kept wet and erosion decreasing. One of the reasons that traditional ways of mechanical weed control practices cannot cultivate conservation tillage is because of the soil disturbances they create; nonetheless, through the limitation of tillage, RRF systems would allow fostering soil and organic matter preservation and carbon sequestration (Cerdeira *et al.*, 2021).

Further, reduced soil disturbance implies enhanced water infiltration and lower surface runoff, more so in augmenting their water use efficiency (Owen *et al.*, 2020). As well, the RRF technology allows on-time implementation of planting operations and harvesting, as there is greater consistent control over the pressure the weeds present. This will assist in enhancing the crop planning and enhancing the profitability and trustworthiness of cotton production complexities. Technology can also be added to integrative pests and diseases management plans, where a cleaner field habitat may be available, and it has potential opportunities to reduce pest habitats and improve scouting views (Chandler *et al.*, 2020).

Although the RRF cotton offers a lot of pluses on the table, there are various limitations that have been emerging due to over-reliance on glyphosate as the single mode of action. The most striking one is the very swift adaptation by the glyphosate-resistant weeds since it has become one of the most important threats to the very sustainability and flexibility of glyphosate-based weed suppression effort. Extensive and repetitive use of glyphosate brings intense selective pressure on the weed's communities leading to the thriving of resistant biotypes such as *Amaranthus palmeri*, *Conyza canadensis* and *Lolium rigidum* (Heap *et al.*, 2024).

Due to increased prevalence of glyphosate-resistant weeds, there is a high risk that weed management can no longer be sustained over time and is not cost-effective. Even though the initial application of glyphosate can kill these resistant individuals, their continued use enables the

survival, and thus, their spread to agroecosystems and the consequent increase in management activities on longer timescales. In this scenario, the *Ipomoea* spp. of the genus morning-glory and *Euphorbia heterophylla* are such tolerant species, and with lack of management, they may outcompete cotton plants and reduce production of cottons (Beckie *et al.*, 2021).

Moreover, it is accompanied by a long-term reduction of the agroecosystem diversity and resilience. An agronomic dependence on glyphosate-focused integrated weed-management schemes is reducing the scope of operation of management to become more vulnerable to ecological demands of an ecosystem. A decline in functional diversity may create conditions of near monoculture, resulting in the development of new pest complexes, revegetation in the composition of weeds, and altering the assemblage of soil microbes, all of which pose a danger to future agricultural sustainability (Owen *et al.*, 2020). Simultaneously, alongside these environmental considerations, the agrochemical industry has been facing the challenges of increasing regulatory pressure and ecotoxicological issues with reference to massive glyphosate application. The increasing publicity and legal action with the environmental monitoring have sparked calls to place glyphosate-based products under the strictest oversight, which may limit their further usage and availability (Benbrook *et al.*, 2023).

## **7. Future Directions**

### **7.1 New Biotechnologies**

The last few decades have witnessed a significant progress in the development of stacked herbicide tolerant cotton lines, like XtendFlex and Enlist that increases the repertoire of herbicides that can be employed in the cotton production systems (Zhang *et al.*, 2023). These cultivars enhance the efficacy of the control of weeds and deter the development of resistance by enabling the use of multiple herbicides with different modes of action (Kumar *et al.*, 2023). The availability of a mixture of different herbicides helps to increase the sustainability of cotton production in the long-term and aids with IWM plans (Bunithan *et al.*, 2023).

Parallel to these, the area of biotechnology has improved significantly in terms of editing of the genome (CRISPR/Cas based) which offers optimism that the cotton may be made resistant to the newer and older herbicides (Nguyen *et al.*, 2024). Genome editing tools are also being researched in order to be able to alter plant architecture, metabolic processes and allelopathy in order to make them more capable of suppressing weeds (Heap *et al.*, 2024). The innovations stand

a good chance of minimizing the spread of the herbicide resistance weeds population and ensuring the viability of the chemical control methods over during the next few decades (Singh *et al.*, 2022).

## **7.2 Alternative Traits**

Biotechnology and breeding efforts are redirecting to provide tolerance to alternative herbicides in other ways than the glyphosate chemistry, which helps to reduce the reliance on single herbicide mode of action (Kumar *et al.*, 2023). The measures enable practicing cotton production in different systems of control over weeds which, except enhancing the degree of flexibility of weed control mechanisms, retard the process of development of the herbicide-resistant types of weeds (Beckie *et al.*, 2021). Combination of the traits to tolerate other herbicides like glufosinate, dicamba and 2, 4-D will help the cotton growers to have a wider range of herbicides that can be used to control some of the hard to-control weed species (Carvalho *et al.*, 2024). This diversification is a very important step to the long-term productivity and support of the sustainable IWM programs (Dille *et al.*, 2022).

## **7.3 Precision Agriculture and Robotics**

Modern precision farming equipment has enabled the user to remove weeds within the field thereby, improving efficiency in herbicide application without compromising the environmental responsibility. Real-time imaging and mapping of weeds allows homing in on the afflicted areas to apply the herbicides only where they are required in Ontario (Bunithan *et al.*, 2023). A high level of accuracy reduces the use of chemicals and ecological imprinting (Valdez *et al.*, 2023).

At the same time, new autonomous robotics suppress or kill weeds in the field, eliminating the problem of herbicide residues (Walsh *et al.*, 2022). The moderate combination of these interventions will have the effect of significantly emasculating herbicide loads without impacting on the control of the weeds, thus positioning itself in the global trends and facilitating the shift to cotton production systems that are characterized by low chemical use and high sustainability.

## **7.4 Biological Weed Control**

Biological control to suppress the growth of weeds; indeed, biological control agent, such as weed-suppressive fungi and bacteria, and allelopathic crop species are viable ways that can be used to manage weeds (Charudattan, 2024). Take the example, certain plant pathogens have the potential to be converted into bioherbicides so that they can be best used to attack the invasive species of the weeds and no harm is caused to crops or other beneficial organisms (Shaner *et al.*, 2020). Incorporation of the biological control in the cotton weed control programs would reduce

the quantity of herbicides and the quality of the soil and result in fewer impacts on the environment. The objects of further study must be founded on the commercial preparations of bioherbicides and discovering synergetic effects of the biological regulator and cultural practices (Mortensen *et al.*, 2022).

### **7.5 Climate-Smart Weed Management**

Global climate change is set to disrupt the weeds within cotton production: it will make different weed species present, take different amounts of time to grow, and even the effectiveness of herbicides will change (Valdez *et al.*, 2023). Raised temperatures, altered rainfall patterns, and increased CO<sub>2</sub> concentration, collectively make the weeds into an even faster growing and spread invasive species (Benbrook *et al.*, 2023). Some of the ways that researchers and farmers are dealing with this is the use of climate-smart weed management which entails planting in different dates, planting cotton varieties that are capable of withstanding the new climate, introduction of cover crops, and switching to conservation tillage. Research should also continue to test varying herbicides under varying weather, just so that we will be able to keep the weeds in line when the weather keeps varying (Charudattan, 2024).

### **7.6 Policy and Research**

One of the most persistent findings in the body of research on sustainable weed management is that farmers always need to be more than theoretically interested in the concept: they need to have a more elaborate and elaborate policy framework and a more intensive program of educating the growers (Beckie *et al.*, 2021). On the policy front, it includes subsidy and regulatory instruments that incentivize those producers who undertake a comprehensive package of weed-control approaches and are consistent with the well-defined best-management guidelines (Norsworthy *et al.*, 2023). Educational programs, in their turn, should emphasize the long-term synergies of using all forms of cultural, mechanical, biological, and chemical methods (Mahoney *et al.*, 2022).

In parallel to the efforts being made in classrooms, long-term field trials hold the key to the evaluation of the resistance-management tactics in different agroecological settings (Peterson *et al.*, 2023). These trials should focus on herbicide package combination, optimal crop rotation, implementing cover crops, tillage regime calibration and experimenting with the use of biological control agents (Charudattan, 2024). The final location in which policy, research and on-farm activities intersect or overlaps is where the weed management regime in the industry will be

expected to withstand an adaptable form of resilience in the face of herbicide resistance evolution (Mortensen *et al.*, 2022).

The extensive use of glyphosate in Roundup Ready Flex cotton has accelerated the emergence of resistant weed species, making integrated management approaches essential. Incorporating chemical, cultural, and mechanical practices can reduce selection pressure and support long-term sustainability of the technology. Table 2 summarizes the main strategies available for resistance management, including their application methods, effectiveness, and possible limitations.

**Table 2. Weed resistance management strategies for RR Flex cotton**

Strategy	Implementation	Effectiveness	Limitations	Citations
Herbicide rotation	Alternating glyphosate with dicamba, glufosinate or residual herbicides	High when diverse MOAs are used, delays resistance	Requires cost, careful planning, and timely applications	Peterson et al. (2023)
Cover cropping	Rye, clover, or vetch to suppress weed emergence	Reduces weed seed bank, improves soil health	May compete with cotton for water/nutrients; extra management needed	Mahoney et al. (2022)
Mechanical control	Cultivation, inter-row hoeing, hand weeding	Effective for escaped weeds and seed bank reduction	Labor-intensive, not feasible on large farms	Ahmed and Saleem (2021)
Gene stacking	Combining glyphosate tolerance with	Highly effective in controlling resistant weeds	Expensive seed cost; risk of resistance shifting	Nguyen et al. (2024)

	glufosinate or dicamba resistance		to multiple herbicides	
Cultural practices	Crop rotation, high plant density, narrow row spacing	Suppresses weed growth and seed production	Requires adaptation of cropping systems	Kumar et al. (2020)

## 8. Conclusion

As it is one of the remarkable changes that have occurred in the background of modern agricultural practices; RRF cotton provides an improved weed control, very minimal plowing requirements and high efficiency. The characteristic allows easy application of herbicides and prefers conservation tillage that increases yield consistency, nutritional status and sustainability of the environment. Nevertheless, this largely and quasi-monofunctional long-term use of glyphosate has led to the emergence and even spread of a glyphosate-resistant forms of weeds and in the weed community to a switch to the more tolerant taxa. These are shifts which undermine the viability and success of the RRF technology and diminish ecological sustainability of cotton cultivation. What is significant therefore is the fact that there is a movement towards a more comprehensive and integrated approach. IWM is the overall management strategy that entails the integration of chemical, mechanical, cultural and biological control mechanisms so as to create some variety of selection pressure and delay the development of resistance to herbicides. IWM enhances the sustainability and flexibility of cotton weed-control programs, by adding and incorporating crop rotation, cover crops and residual herbicides, and site-specific guidance. In the future, efforts should be put in high ranking in innovation of herbicide-tolerant stacking of traits, gene-editing technologies, precision-agriculture system, and evolution of environmentally benign management. Development of stewardship programs and education of the farmers should also follow a logical policy framework to sustain long-term productivity and ecological stability. The final stand of formulating sustainable weed management in cotton systems will rely on the capabilities to diversify adaptable weed management, with the aspect of balancing the interests of crop production with the environment.

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