

REVIEW ARTICLE

Herbicide-resistant weeds in Greek rice cultivation systems: A Review of the status, drivers, and Integrated Management Implications

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Summary Herbicide resistance has emerged as a major constraint to sustainable rice (*Oryza sativa* L.) production in Greece, where intensive water-seeded monoculture systems rely heavily on chemical weed control. This review synthesizes current knowledge on rice production systems, herbicide use patterns, and the evolution of herbicide-resistant weeds in Greek agroecosystems. Rice cultivation is concentrated in northern Greece under flooded conditions that favor the proliferation and persistence of competitive weed species. The dominant resistant weeds include *Echinochloa* spp., *Cyperus difformis*, weedy rice (red rice) and *Leptochloa fusca* subsp. *fascicularis*. Resistance has been documented to multiple herbicide modes of action, including propanil, acetolactate synthase (ALS) inhibitors, acetyl-CoA carboxylase (ACCase) inhibitors, synthetic auxins, and glyphosate, with increasing occurrence of cross- and multiple-resistant biotypes. Resistance evolution is driven by prolonged herbicide reliance, continuous monoculture, seedbank persistence, and dispersal through irrigation networks. The review further evaluates species-specific resistance mechanisms and highlights integrated weed management strategies based on preventive, tactical, and long-term Best Management Practices. This baseline assessment provides a consolidated reference for researchers, advisors, and policy-makers and supports the development of coordinated resistance management frameworks aligned with EU Integrated Pest Management principles and the specific challenges of Greek rice production.

Additional keywords: ALS inhibitors, cross-resistance, integrated weed management, paddy fields, resistance mechanisms, water seeded rice

1. Introduction

Rice cultivation in Greece is geographically concentrated in a few major delta and basin systems, namely the Axios–Aliakmonas–Gallikos Delta, the Strymonas/Serres Basin, and smaller production areas in Kavala/Drama and western Greece in Amvrakikos region, (Vasilakoglou *et al.*, 2018). These regions account for the national rice production and are characterized by intensive, monocultural, flooded paddy systems. Such production conditions create an ideal environment for the evolution and estab-

lishment of herbicide-resistant weed populations, turning Greek rice agroecosystems into key hotspots of herbicide resistance (Vasilakoglou *et al.*, 2000; Kaloumenos *et al.*, 2013a; Kaloumenos *et al.*, 2013b).

Over the past three decades, numerous cases of resistance have been documented among the dominant weed species, including *Echinochloa phyllopogon*, *E. oryzicola*, *E. crus-galli*, *E. colona*, *Cyperus difformis*, *Oryza sativa*, and *Leptochloa* sp. (Heap, 2026). The weed flora in Greek paddies is dominated by highly fecund and persistent species, such as *Echinochloa* spp., *Cyperus difformis*, and weedy/red rice (*Oryza sativa* f. *spontanea*); those species biology, including high seed output, prolonged seed persistence, and adaptability to alternating moisture regimes, amplifies the risk of resistance selection under repeated use of narrow-spec-

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trum herbicides (Damalas *et al.*, 2008; Osca *et al.*, 2021).

A special perspective on herbicide resistance is necessary in Greece since water-seeded rice systems in the country present a unique combination of agronomic, ecological, and socio-economic features that distinguish them from upland or Asian and American rice systems. Key contributors in the accelerated evolution of herbicide resistance in Greek rice systems include the long-term reliance on a limited range of herbicide modes of action, which has created a highly selective environment favoring the proliferation of resistant biotypes, minimal crop rotation, and the occurrence of weed mimicry, particularly in *Echinochloa phyllopogon* (Papapanagiotou *et al.*, 2025a). Additional drivers involve the re-infestation of paddies through irrigation and drainage canals, which act as reservoirs of resistant biotypes, and the biological stability and persistence of resistant phenotypes, which allow their propagation across seasons without significant fitness penalties.

Greek specific constraints, including the limited herbicide toolbox, EU regulatory restrictions, and environmental protection requirements, shape both the choice and timing of chemical interventions, making stewardship and Best Management Practices (BMPs) context-dependent. Evidence from other regions shows that BMPs succeed only when tailored to local biological, chemical, and socio-economic conditions (Norsworthy *et al.*, 2012).

The present baseline report consolidates available knowledge on the geographic distribution of resistance, mechanisms underlying herbicide adaptation, agronomic drivers, and potential management strategies in Greek rice production systems. By synthesizing these data, the report aims to provide a comprehensive foundation for understanding current resistance dynamics and informing future integrated weed management interventions. This work has been conducted within the framework of the Hellenic Foundation for Research & Innovation (H.F.R.I) research project named ARTS-Rice project

(www.arts-rice.gr), which aims to enhance sustainable rice production in the Mediterranean through integrated weed management and the mitigation of herbicide resistance.

2. Rice production systems in Greece

Production systems

Rice cultivation in Greece is concentrated primarily in Central Macedonia, including the prefectures of Thessaloniki, Imathia, and Pella, which collectively represent the largest production zone (>70%) and focus on intensive commercial varieties, both japonica and indica types. The second major area is the Serres/Strymonas Basin (~20% of national production), while Eastern Macedonia–Thrace, encompassing Kavala and Drama, supports smaller but historically significant areas for the emergence and study of herbicide-resistant weeds. Finally, the Arta/Amvrakikos region represents a minor production area. Across these regions, rice agroecosystems are highly specialized, with heavy alluvial soils, permanent irrigation infrastructure, and intensive management practices.

Continuous rice monoculture dominates Greek rice cultivation, with minimal crop rotation and uniform management practices (Papapanagiotou *et al.*, 2025a). Soils are generally heavy-textured and fertile, allowing high yields under flooded conditions. Key weed species that thrive under these agroecological conditions include *Echinochloa* spp., *Cyperus difformis*, *Alisma plantago-aquatica*, and weedy rice populations, many of which have developed herbicide resistance over time (Vasilakoglou *et al.*, 2018). The combination of monoculture, uniform herbicide application, and the stability of resistant phenotypes has contributed to the persistence and expansion of problematic weed populations.

Herbicide use

Weed control strategies rely heavily on a rather narrow portfolio of herbicides, which

has intensified selection pressure and contributed to resistance evolution. During the last 5 years, commonly used modes of action; data retrieved from the Greek Ministry of Rural Development and Food's Plant Protection Products database on January 8, 2026. The list is as follows:

- ALS inhibitors (Group 2): penoxsulam, bispyribac-sodium, halosulfuron-methyl and imazamox; only in Clearfield systems
- ACCase inhibitors (Group 1): cyhalofop-butyl, profoxydim, propaquizafop, and clethodim; only in Provisia systems
- Auxinic herbicides (Group 15): MCPA, triclopyr, florypyrauxifen
- PS-II Inhibitors (Group 5): bentazone
- EPSPS inhibitors (Group 9): glyphosate
- Microtubule Polymerization Inhibitor (MPI) (Group 3): pendimethalin
- DOXP inhibitor (Group 12): clomazone
- HPPD inhibitor (Group 27): benzobicyclon

In addition to fully authorized plant protection products, several herbicides have been authorized in Greek rice systems under emergency authorizations, as permitted under Article 53 of Regulation (EC) No 1107/2009 (European Parliament and Council of the European Union, 2009). These include benzobicyclon, profoxydim, propanil, quinclorac, and pretilachlor, all granted for periods not exceeding 120 days. The recurring reliance on such emergency authorizations reflects the structural limitations of the fully registered herbicide portfolio in Greek rice and highlights the regulatory vulnerability of the sector's weed management toolbox, particularly in the context of escalating herbicide resistance.

3. Herbicide resistance in rice

Propanil Resistance

Historically, the first documented cases of herbicide resistance in Greek rice oc-

curred in the 1980s with *Echinochloa crus-galli* exhibiting reduced susceptibility to propanil (Giannopolitis and Vassiliou, 1989). Metabolic detoxification mechanisms were identified as key contributors to early resistance, signaling the vulnerability of Greek rice systems to adaptive responses among dominant weeds (Giannopolitis and Vassiliou, 1989; Vasilakoglou *et al.*, 2000). These early observations highlighted the limitations of relying solely on a single mode of action for weed control and set the stage for future resistance monitoring.

ALS Resistance Establishment

This emergence coincided with the intense reliance on ALS inhibitors (*i.e.* during the 2000-2010 period), reflecting the direct link between herbicide use patterns and the evolution of target-site resistance. As such, ALS-inhibitor resistance became increasingly widespread. Resistance was confirmed in *Echinochloa oryzoides* and *E. phyllopogon* (Damalas *et al.*, 2006, 2008), while *Cyperus difformis* developed ALS resistance via specific point mutations in the ALS gene (Ntoanidou *et al.*, 2016).

Cross- and Multiple Resistance

Between 2010 and 2020, multiple cases of cross-resistance to acetolactate synthase (ALS)-inhibiting herbicides were confirmed in Greek rice agroecosystems, involving active ingredients such as penoxsulam, bispyribac-sodium, and imazamox. During the same period, the emergence of acetyl-CoA carboxylase (ACCase) inhibitor resistance was also reported, further constraining chemical control options (Damalas *et al.*, 2006; Vasilakoglou *et al.*, 2018). In addition, documented herbicide antagonistic interactions reduced field-level efficacy, exacerbating control failures. Collectively, these developments reflect the cumulative effects of prolonged and repetitive herbicide selection pressure and expose the structural limitations of conventional herbicide rotation schemes in managing genetically diverse and adaptable weed populations.

Since 2020, resistance patterns have be-

come increasingly complex, with stacked and multi-mechanism resistance phenotypes now being reported. Notably, multiple resistance to ALS inhibitors, ACCase inhibitors, and auxinic herbicides has been identified in *Echinochloa phyllopogon* populations (Papapanagiotou *et al.*, 2025a), while glyphosate resistance has been documented in *Echinochloa colona* (Travlos *et al.*, 2020). Importantly, resistant biotypes appear to exhibit no detectable fitness penalties under non-treated conditions (Papapanagiotou *et al.*, 2023), facilitating their persistence, spread, and competitive dominance within rice production systems.

This advanced resistance phase represents a critical challenge for sustainable weed management in Greek rice systems and underscores the urgent need for integrated weed management (IWM) approaches. Such strategies must move beyond herbicide-centric paradigms and incorporate chemical diversification, cultural practices, ecological regulation, and system-level redesign to effectively mitigate resistance evolution and ensure long-term agroecosystem resilience.

4. Weed species-by-species resistance profile to herbicides in rice

Echinochloa phyllopogon (late water-grass)

Resistance status. *Echinochloa phyllopogon* has become one of the most problematic weed species in Greek rice systems due to its widespread and robust resistance profile. ALS inhibitor resistance is strong and prevalent across major rice-growing zones (Vasilakoglou *et al.*, 2018), while ACCase resistance has also been confirmed in multiple field populations (Papapanagiotou *et al.*, 2025a). Resistance to auxinic herbicides, including dicamba and MCPA, has emerged more recently (Papapanagiotou *et al.*, 2025a). Greece represents one of the earliest European cases where multiple resistance to ALS, ACCase, and auxinic herbicides has been documented (Papapanagiot-

ou *et al.*, 2025a).

Global studies indicate emerging resistance and reduced sensitivity of *Echinochloa* species to florypyrauxifen-benzyl, driven by target-site mutations and metabolic detoxification mechanisms (Zhang *et al.*, 2023; Deng *et al.*, 2025). Although only empirical reports of reduced efficacy currently exist in Greece, the confirmation of similar metabolic resistance mechanisms in Greek *Echinochloa* populations suggests a high risk of cross-resistance and necessitates proactive monitoring to ensure long-term herbicide efficacy.

Mechanism. Resistance in *E. phyllopogon* is driven by a combination of target-site and non-target-site mechanisms. ALS target-site mutations, including Trp574 and Pro197 variants, are commonly found. Additionally, enhanced metabolic detoxification, likely mediated through cytochrome P450 enzymes, contributes to herbicide breakdown. Importantly, these resistant biotypes exhibit no measurable fitness penalty, allowing them to persist and spread under continuous selection pressure.

Echinochloa oryzicola

Resistance status. *Echinochloa oryzicola* is considered one of the most noxious weeds in rice fields in Northern Greece (Kaloumenos *et al.*, 2013b). Several studies have been conducted in order to confirm resistance to ALS-inhibiting herbicides. Specifically, it has been found that *E. oryzicola* indicates cross resistance to penoxsulam, bispyribac-sodium, imazamox, foramsulfuron, nicosulfuron and rimsulfuron. It is suggested that cross resistance to ALS herbicides is imposed by continuous use of them and highlights the herbicide selection pressure caused by rice monoculture.

Mechanism. *Echinochloa oryzicola* exhibits well-documented ALS inhibitor resistance, primarily associated with the Trp-574-Leu point mutation (Kaloumenos *et al.*, 2013b).

***Echinochloa crus-galli* (barnyard grass)**

Resistance status. *Echinochloa crus-galli* was the first Greek rice weed reported to develop herbicide resistance, specifically to propanil (Giannopolitis and Vassiliou, 1989; Vasilakoglou *et al.*, 2000). Subsequent surveys have noted reduced susceptibility to ALS-inhibiting herbicides, suggesting ongoing adaptation under continued selection pressure.

Mechanism. Metabolic resistance, potentially involving enhanced detoxification pathways, is likely responsible for reduced herbicide sensitivity (Kanas, 2020).

***Echinochloa colona* (jungle rice)**

Resistance status. Glyphosate resistance has been confirmed in *E. colona* populations (Travlos *et al.*, 2020). While this species primarily affects levee and canal systems, its seeds can be transported via water into paddies, leading to reinfestation and potential establishment within cultivated fields.

Mechanism. No EPSPS target-site mutations have been detected, suggesting that resistance is driven by metabolic mechanisms that degrade glyphosate or reduce its uptake.

***Echinochloa oryzoides* (early watergrass)**

Resistance status. A major challenge with *E. oryzoides* is its morphological similarity to cultivated rice during early growth stages (Altop *et al.*, 2014). This mimicry allows seedlings to escape pre-emergence and early post-emergence herbicide applications, resulting in significant contributions to the soil seedbank and making management particularly difficult. Early signs of AC-Case tolerance were also observed in some populations (Damalas *et al.*, 2006), suggesting the potential for future multiple-resistance development.

Mechanism. No further evidence is available regarding the underlying resistance mechanisms implying that a genetic-based classification between tolerant and susceptible

accessions cannot be defined (Altop *et al.*, 2014).

High-Risk Traits in *Echinochloa* spp. *Echinochloa* spp. possess a set of biological traits that facilitate resistance evolution and persistence. The key biological traits that confer high resistance risk to each *Echinochloa* species present in Greek rice systems are summarized in Table 1.

***Cyperus difformis* (rice sedge)**

Resistance status. Control of rice sedge has declined sharply in areas where ALS herbicides dominate weed management programs. This trend underscores the necessity for diversified weed control strategies combining chemical, cultural, and mechanical measures to prevent further expansion of resistant populations.

Mechanism. ALS resistance in *Cyperus difformis* is widely documented across Greece (Ntoanidou *et al.*, 2016; Vasilakoglou *et al.*, 2018). Point mutations at Pro197 and Trp-574-Leu confer cross-resistance to multiple ALS-inhibiting herbicides, complicating conventional control approaches. Cross-resistance in Greek populations extends specifically to azimsulfuron and halosulfuron-methyl: Ntoanidou *et al.* (2016) demonstrated that applications of up to 64 times the recommended field rate of both herbicides failed to reduce growth of resistant *C. difformis* populations from the Thessaloniki area by 50%, while a susceptible reference population was controlled at one quarter of the recommended rate. This high-level cross-resistance across multiple ALS-inhibiting active ingredients further restricts the chemical control options available for sedge management in Greek rice and reinforces the need for herbicide diversification incorporating non-ALS modes of action such as bentazone, benzobicyclon, and florypyrauxifen-benzyl (Tehranchian *et al.*, 2015).

Weedy rice (red rice)

Resistance status. Weedy rice exhibits variable sensitivity to imazamox (Vasilakoglou

Table 1. Biological traits of *Echinochloa* spp. that increase the risk of herbicide resistance evolution and persistence in Greek rice paddies.

Species	Trait Increasing Resistance Risk
<i>E. phyllopogon</i>	High fecundity, prolonged emergence period, extensive genetic variability, water-dispersed seeds facilitating movement across paddies
<i>E. oryzoides</i>	Rice mimicry during early growth, allowing escape from pre- and early post-emergence management, contributes to large soil seedbanks
<i>E. crus-galli</i>	Rapid metabolic adaptation, enabling early development of herbicide tolerance and potential cross-resistance
<i>E. colona</i> <i>E. oryzicola</i>	Thrives in levee and canal margins, seeds easily invade paddies via water movement, facilitating regional dispersal

and Dhima, 2005). Although full resistance has not yet developed in Greek rice fields, the species poses a high evolutionary risk due to potential gene flow with imidazolinone-tolerant rice varieties cultivated elsewhere. If such gene transfer occurs, rapid emergence of resistant weedy rice could pose serious threats to production, necessitating close monitoring and proactive management strategies.

Mechanism. The resistance mechanisms include: a) Target-site mutations: ALS (acetolactate synthase) gene in weedy rice often harbors amino acid substitutions. For example, in studies from other regions, the S653N substitution a) key mutation conferring imidazolinone resistance) has been found (Unan *et al.*, 2024); b) Gene flow: The same S653N mutation is found in IMI-resistant cultivated rice, suggesting that resistance in weedy rice may arise via cross-pollination (Unan *et al.*, 2024); c) Non-target site mechanisms: Some resistant weedy rice individuals do not show ALS mutations, which implies possible metabolic resistance, or other mechanisms (e.g., enhanced herbicide detoxification) (Unan *et al.*, 2024).

Evolution through selection pressure: Repeated use of imazamox (IMI) creates strong selective pressure, favoring both spontaneous mutants and gene flow events over time.

***Leptochloa fusca* spp. *fascicularis* (bearded sprangletop)**

Resistance status. Populations of *Leptochloa fusca* spp. *fascicularis* have developed resis-

tance mechanisms to ALS-inhibiting herbicides in rice monoculture systems in northern Greece (Papapanagiotou *et al.*, 2025b). It has been spotted on cross-resistance to ACCase inhibitors, including profoxydim, propaquizafop, cyhalofop + penoxsulam ACCase inhibitor cross resistance restricts the efficacy of available chemical control methods bearded sprangletop in rice cultivation.

Mechanism. Resistance to *Leptochloa fusca* spp. *fascicularis* is related to target-site mutation of the ACCase enzyme. Specifically, this point mutation leads to substitution of tryptophan (Trp) by cysteine at position 1999 in the CT domain of the ACCase gene (Papapanagiotou *et al.*, 2025a).

5. Management implications and recommendations

Herbicide resistance management

Chemical control strategies must evolve to mitigate resistance. Immediate diversification of herbicide modes of action is critical, incorporating compounds such as floryauxifen, early-residual products (such as clomazone) and HPPD inhibitors (such as benzobicyclon). Herbicide rotation plans should be informed by population-specific resistance profiles, alternating ALS, ACCase, and auxinic herbicides where effective. In areas where ALS resistance is confirmed, ALS herbicides should be avoided to prevent further selection pressure.

Introduction of new herbicides in rice systems

The increasing prevalence of herbicide-resistant weed populations in rice has intensified the need for new herbicide sites of action that can be integrated into existing weed management programs. In water-seeded rice systems, long-term reliance on acetolactate synthase (ALS) and acetyl-CoA carboxylase (ACCase) inhibitors has resulted in widespread resistance among key species such as *Echinochloa* spp., *Cyperus difformis*, and other aquatic weeds.

In response to these challenges, the recent introduction of benzobicyclon, a 4-hydroxyphenylpyruvate dioxygenase (HPPD) inhibitor, and florpyrauxifen-benzyl, a synthetic auxin of the arylpicolinate group, has expanded the herbicide portfolio available for rice production. These herbicides provide alternative modes of action, improved crop selectivity under flooded conditions, and new opportunities for rotating and diversifying chemical weed control strategies, thereby supporting integrated approaches to herbicide resistance management in modern rice systems (Norsworthy *et al.*, 2012; Duy *et al.*, 2018; Poole, 2020).

Benzobicyclon. Benzobicyclon introduces a mode of action distinct from ALS and ACCase inhibitors, which dominate weed control programs in Greek rice systems. Its importance lies in providing an effective alternative for managing weed populations resistant to these commonly used herbicide groups (Papapanagiotou *et al.*, 2025a). From a resistance-management perspective, benzobicyclon is particularly valuable for controlling ALS-resistant sedges, broad-leaf weeds, and *Echinochloa* spp., and has also demonstrated some activity against resistant weedy rice populations. Its integration into rice weed management programs allows rotation of herbicide sites of action and reduces selection pressure on ALS and ACCase inhibitors, provided it is used within diversified chemical and cultural strategies (Liu *et al.*, 2021).

Florpyrauxifen-benzyl. Florpyrauxifen-benzyl represents a novel auxinic chemistry for rice weed management. Unlike older synthetic auxins, florpyrauxifen-benzyl primarily interacts with the AFB5 auxin receptor, resulting in a distinct physiological response in susceptible weeds (Epp *et al.*, 2016).

The herbicide is characterized by excellent crop selectivity in rice, allowing both pre-emergence and post-emergence applications without significant phytotoxicity when applied according to label recommendations (Epp *et al.*, 2016). This selectivity, combined with systemic activity, enables effective control of a broad spectrum of problematic weeds in flooded rice systems, including *Echinochloa* spp., *Cyperus difformis*, *Schoenoplectus* spp., *Heteranthera* spp., and *Alisma* spp., many of which have developed resistance to ALS inhibitors.

From a resistance-management standpoint, florpyrauxifen-benzyl offers a critically important alternative site of action for Mediterranean rice systems heavily reliant on ALS and ACCase inhibitors. Its use in sequential or rotational programs, either alone or in combination with other herbicides, contributes to diversified selection pressure and helps delay further resistance evolution. However, indications of reduced sensitivity to florpyrauxifen-benzyl have been documented in *Echinochloa* spp. populations in other rice-producing regions. Lim *et al.*, (2021) and Takano *et al.*, (2023) reported that CYP450-mediated metabolic detoxification can confer reduced sensitivity to florpyrauxifen-benzyl in *Echinochloa* populations with a prior history of ALS inhibitor exposure. This finding is of particular relevance to Greek rice systems, where CYP450-mediated metabolic resistance to ALS and ACCase inhibitors has already been confirmed in *E. phyllopogon* populations (Papapanagiotou *et al.*, 2025a), raising the possibility that cross-resistance to florpyrauxifen-benzyl may emerge under continued selection pressure.

This cross-resistance risk is particularly relevant in Greek rice systems, where repeated exposure to ALS inhibitors has progres-

sively selected for enhanced CYP450-mediated detoxification, the same mechanism already confirmed in *E. phyllopogon* populations from Greek rice fields (Papapanagiotou *et al.*, 2025a). Given the critical role of florypyrauxifen-benzyl in modern weed management, its long-term viability depends on a shift away from exclusive reliance toward its strategic integration within diversified management frameworks. To safeguard its efficacy and ensure the sustainability of rice production, it is essential to combine this chemistry with alternating modes of action and robust cultural control tactics (Wright *et al.*, 2021). Such proactive stewardship is not merely a recommendation but a necessity to prevent the onset of resistance and preserve this vital tool for future use.

Combination of chemical control with rotational crops

In Greek rice systems, when rotational crops are used, the following could be applied: a) Crop rotation with cotton and the use of soil-applied herbicides (benfluralin, pendimethalin) or post-emergence grass herbicides (propaquizafop, quizalofop, cycloxydim, clethodim). These herbicides have a different mode of action than ALS inhibitors and therefore offer effective alternatives against imazamox-resistant populations; b) Crop rotation with maize, combined with soil-applied herbicides either single or in mixtures such as (dimethenamid + terbuthylazine, or pethoxamid + terbuthylazine or mesotrione + terbuthylazine or sulcotrione + terbuthylazine). Application of pre-emergence herbicides with different modes of action also helps manage ALS-resistant populations; c) Crop rotation with cereals, particularly barley (*Hordeum vulgare* L.), is an effective complementary strategy for managing herbicide-resistant weeds in Greek rice-based systems, especially in coastal and saline soils where barley is well adapted. The cereal phase disrupts the life cycle of rice-adapted weeds, reduces the soil seedbank of *Echinochloa* spp. and weedy rice. The shift to autumn sowing further alters weed emergence patterns and enables

the use of cultural practices that contribute to seedbank depletion. From a resistance-management perspective, cereals provide both chemical diversification and ecological disruption, reducing selection pressure on rice herbicides and limiting the carryover of resistant weed populations into subsequent rice crops (Buhler *et al.*, 1997; Beckie, 2006).

Herbicide-Tolerant Rice Systems. The management of herbicide-resistant weeds in water-seeded rice systems has been particularly challenging in Mediterranean environments, where continuous rice monoculture, limited crop rotation options, and prolonged flooding constrain the diversity of available weed control tactics. Under these conditions, herbicide-tolerant (HT) rice systems have emerged as important tools within integrated weed management frameworks, especially for controlling weedy rice (*Oryza sativa* f. *spontanea*) and herbicide-resistant grass weeds such as *Echinochloa crus-galli*.

The Clearfield® Rice System. The Clearfield® rice system, based on tolerance to imidazolinone (IMI) herbicides, was first commercialized in the early 2000s and represents a major milestone in rice weed management. Clearfield rice varieties are non-genetically modified and were developed through induced mutation and conventional breeding, carrying point mutations in the acetolactate synthase (ALS) gene that confer resistance to IMI herbicides such as imazamox and imazethapyr (Tan *et al.*, 2006). These mutations typically involve amino acid substitutions at key positions of the ALS enzyme (e.g. Ala122, Ser653), reducing herbicide binding without compromising crop fitness.

In Mediterranean rice systems, including Greece, Clearfield technology has enabled effective post-emergence control of weedy rice populations and *Echinochloa* species that are difficult or impossible to manage selectively in conventional rice. Yield stability and harvest quality improvements following Clearfield adoption have been widely documented (Burgos *et al.*, 2008;

Kaloumenos *et al.*, 2013b). However, the intensive and repeated use of IMI herbicides has also resulted in rapid selection pressure, leading to the evolution of IMI-resistant weedy rice through both target-site resistance and gene flow from Clearfield cultivars (Busconi *et al.*, 2012; Goulart *et al.*, 2014).

Hybridization between Clearfield rice and weedy rice has been reported in several rice-growing regions within only a few years of adoption, highlighting the need for strict stewardship (Shivrain *et al.*, 2007; Norworthy *et al.*, 2012). In Mediterranean environments, where water-seeded systems favor synchronous emergence and flowering, the risk of outcrossing may be particularly high if volunteer rice and escaped weedy rice plants are not rigorously controlled. Consequently, Clearfield rice cannot be considered a standalone resistance solution but rather a transitional tool that must be embedded within rotation schemes, non-ALS herbicide use, and cultural practices such as stale seedbeds and volunteer management.

The Provisia® Rice System. To address the increasing prevalence of IMI-resistant weedy rice and grass weeds, the Provisia® rice system was developed as a complementary HT technology. Provisia rice varieties are tolerant to ACCase-inhibiting herbicides (Group 1), particularly cycloxydim, and were likewise developed through conventional breeding rather than genetic engineering (Camacho *et al.*, 2019). Resistance is conferred by a specific point mutation in the ACCase enzyme (Ile1781→Leu), which reduces herbicide sensitivity while maintaining normal lipid biosynthesis in the crop.

The Provisia system provides effective control of IMI-resistant weedy rice, *Echinochloa* spp., and other problematic grasses, offering a critically needed alternative mode of action in rice systems dominated by ALS resistance. Field evaluations have demonstrated high levels of efficacy when cycloxydim is applied sequentially at early growth stages of target weeds, particularly under low-stress crop conditions (Webster *et al.*, 2021).

In Greece, the recent introduction of Provisia rice represents an important opportunity to diversify chemical weed control in water-seeded rice, where ACCase inhibitors were previously unavailable for selective in-crop use (Papapanagiotou *et al.*, 2025a). However, as with Clearfield, the sustainability of Provisia depends on strict stewardship. ACCase resistance in grass weeds is well documented globally, and over-reliance on cycloxydim would likely result in rapid resistance evolution if used continuously or without integration of residual herbicides and non-chemical tactics (Webster *et al.*, 2021).

Stewardship and integration in rice systems. International experience clearly demonstrates that HT rice systems extend their useful lifespan only when alternated with each other and with conventional rice production. In Greek rice systems, best management practices include rotating Clearfield and Provisia technologies across years, avoiding consecutive planting of the same HT trait, integrating pre-emergence residual herbicides with different modes of action, and enforcing zero tolerance for volunteer rice and weedy rice escapes.

A rotational approach combining Clearfield and Provisia systems, complemented by crop rotation where feasible (e.g. maize, cotton, winter cereals), is particularly relevant for Greece, where rice is often grown continuously in deltaic environments. Such alternation reduces selection pressure on both ALS and ACCase target sites and limits the accumulation of resistant weedy rice biotypes. Importantly, HT rice systems must remain embedded within broader integrated weed management strategies that include water management, competitive cultivars, precise fertilization, and mechanical or manual removal of escapes.

Overall, Clearfield and Provisia technologies represent powerful but finite tools for resistance management in Mediterranean water-seeded rice. Their value lies not in replacing integrated weed management, but

in restoring chemical diversity and enabling strategic rotations that delay resistance evolution while maintaining crop productivity.

Preventive measures and strategies

The use of clean, certified, and weed-free crop seed is one of the most cost-effective and preventive strategies against the introduction and spread of weedy rice (*Oryza sativa* f. *spontanea*) and other problematic weed species. In Mediterranean rice ecosystems, contaminated seed lots represent a primary vector for herbicide resistance spread and weed re-infestation, especially where farmers rely on saved seed. Studies across Greece, Italy, and Spain have shown that even minimal contamination (<1%) can perpetuate weedy rice populations for multiple seasons, due to seed dormancy and genetic mimicry (Ferrero *et al.*, 2021).

Avoiding contamination of rice fields: importance of using clean seed. The weed spreads from contaminated to clean areas through infested seed. Animals and machinery can also disperse wild rice. Some national laws allow small numbers of wild rice seeds in certified seed lots. Early detection and prevention of establishment are the first steps to reduce infestation and spread, especially in direct-seeded systems. Systematic monitoring is essential to prevent invasion and to enable timely management. Some farmers use saved seed from the previous year, which is often contaminated. Piveta *et al.* (2021) estimated that sowing seed contaminated with just 2 red rice seeds per kg can, after three years, produce 10 kg of red rice seed per acre. In Sri Lanka (Chauhan *et al.*, 2014), using clean seed increased yield by 29–41% compared to farmer-saved contaminated seed.

Harvest, drying, and storage sanitation. Physical contamination can also occur post-harvest, when weedy rice seeds are inadvertently mixed during combine harvesting, drying, or storage. Cleaning harvesters, transport augers, and storage bins between rice varieties or fields is critical. Studies in

the U.S. Midsouth (Norsworthy *et al.*, 2013) show that combines not cleaned between fields can transfer up to 10,000 weedy rice seeds per hectare. European best practices now recommend a cleaning protocol involving (i) removal of residual grain from headers and elevators, (ii) operation of the threshing system for 5–10 minutes to expel residual seeds, and (iii) physical washing of key components between seed lots (Gómez de Barreda *et al.*, 2021). Additionally, segregated storage and drying facilities prevent the mixing of different seed lots. In Italy and Spain, regional cooperatives have adopted traceability systems for drying and packaging to maintain seed purity (Vidotto *et al.*, 2021; Osca *et al.*, 2021).

Use of false seeding to manage weeds in paddy fields

Greek rice producers have been able to voluntarily participate in the “false seeding” program under the CAP Rural Development Program, receiving financial support of €200/ha/year. Beneficiaries who opt in are required to implement false seeding (preparation of a seedbed without actual rice sowing) for five consecutive years across all enrolled rice plots. After standard pre-sowing practices, certain obligations must be followed: Flood the plots lightly in April (about one month before normal rice sowing, depending on the region) to stimulate weed seed germination; Drain the water into nearby water bodies (rivers, wetlands, drainage canals); Mechanically destroy the emerged weeds by surface soil treatment before rice sowing.

False seeding can effectively control both resistant and *Ammannia* spp., sedges (Ferrero *et al.*, 2021). Proper seedbed preparation, rational fertilizer use, competitive variety selection, dense sowing, and proper water management enhance the efficiency of false seeding. Using certified weed-free rice seeds is equally important to prevent introducing new weeds. Pre-plant soil treatment to 3–4 cm depth when weed seedlings have 2–3 leaves further improves false seeding efficiency. Deep plowing every 3–4

years during primary field preparation helps bury weed seeds to depths where they cannot germinate or rot due to excess moisture. Although effective against red rice and other grasses, false seeding has some challenges: a) Rice sowing occurs 2–3 weeks later than normal, reducing the feasibility of long-season, high-yielding varieties, and decreasing yields by 100–200 kg/ha. Yield is also affected by soil-climatic conditions and agricultural practices; b) Increased water supply duration by irrigation authorities raises water costs; c) It is difficult to implement on wet, heavy soils.

6. Knowledge gaps, monitoring needs, Best Management Practices

Knowledge Gaps

Despite extensive research documenting herbicide resistance in Greek rice systems, several critical knowledge gaps remain. Firstly, there is no national resistance monitoring scheme, which limits the ability to detect emerging resistant populations in a timely manner or to coordinate mitigation efforts across regions. Secondly, systematic molecular characterization for ACCase and auxinic and to a lesser extent of ALS inhibitor resistance remains rather limited, constraining our understanding of the specific mutations, metabolic pathways, and potential for cross-resistance. Thirdly, high-resolution mapping of soil seedbanks is largely absent, leaving uncertainties regarding the spatial distribution and density of resistant seeds within and between paddies. Finally, long-term datasets evaluating fitness costs of resistant biotypes across diverse environmental conditions are lacking, which hampers predictive modeling of resistance spread and the development of sustainable management strategies.

Monitoring Priorities

To address the aforementioned knowledge gaps, the following monitoring activities should be prioritized which are essential for establishing a coordinated, evidence-

based approach to herbicide resistance management in Greece:

- Annual in-field and regional screening in high-risk regions such as the Thessaloniki Delta region to track emerging resistance trends.
- Molecular diagnostics targeting *Echinochloa* spp. to identify key ALS, AC-Case, and auxinic resistance mutations, enabling early detection and informed management decisions.
- Canal network surveillance to monitor the spread of glyphosate-resistant *E. colona* and other water-dispersed weed populations, which serve as major reinfestation pathways.
- Seedbank resistance mapping to quantify the density, distribution, and temporal dynamics of resistant seeds, supporting strategic interventions at field and landscape scales.

Best Management Practices

Considering the reviewed status, gaps and challenges, Best Management Practices (BMP) that could be applied at various stages of the crop and represent various targets in the Greek rice systems, incorporate preventive, tactical and long-term strategies (Table 2). In particular, preventive measures, including assessment of weed biology (BMP 1), multifaceted weed management strategies (BMP 2), maintenance of weed-free fields (BMP 3), use of certified weed-free seed (BMP 4), and routine scouting (BMP 5), are essential to reduce initial weed pressure and prevent the introduction of resistant biotypes such as *Echinochloa* spp. and weedy rice. Tactical BMPs, encompassing the use of herbicides with multiple modes of action (BMP 6), adherence to labeled application rates (BMP 7), and integration of crop competitiveness and cultural control measures (BMP 8), target early-season escapes, optimize herbicide efficacy, and minimize selection for resistance. Long-term measures, including mitigation of weed seed movement and crop residue management (BMP 9), harvest-time seed management

Table 2. A detailed concept of an Integrated Weed Management guide linking rice growth stages to key Best Management Practices (BMPs) for sustainable weed control and herbicide resistance management in Greek rice systems.

Rice Growth Stage	Preventive BMPs (BMP 1–5)	Tactical BMPs (BMP 6–8)	Long-Term BMPs (BMP 9–11)	Key Notes / Targets
Pre-Planting / Field Prep	Start clean (stale seed-bed, preplant flooding) Ensure weed-free seed (certified seed)	—	—	Reduce initial weed pressure; prevent introduction of resistant seeds. Target <i>Echinochloa</i> and weedy rice first flushes.
Sowing / Early Emergence (0–3 leaf)	—	Apply PRE herbicides with multiple MOAs (BMP 6) Follow label rates and proper calibration (BMP 7)	—	Ensure early weed control; avoid under-dosing and late applications that select for resistance.
Seedling / Vegetative Growth (3–5 leaf)	—	Sequential POST herbicides (multiple MOAs) Apply at optimal growth stage	—	Maintain selective pressure, prevent escapes; monitor weed size and coverage.
Canopy Development / Mid-Season	—	Competitive cultivars, narrow row spacing, water management (BMP 8)	Weed seed movement mitigation, residue management begins (BMP 9)	Reduce late-season weed growth and suppress seed set.
Heading / Pre-Harvest	—	—	Harvest-time management (BMP 10: header adjustments, chaff collection)	Prevent seed return to soil seedbank; reduce spread of resistant biotypes.
Post-Harvest / Field Rest	—	—	Post-harvest flooding, winter cover crops, field border management (BMP 11)	Destroy remaining seeds, reduce reinfestation; coordinate with neighboring farms.

(BMP 10), and management of field borders to prevent reinfestation (BMP 11), are crucial to suppress late-season weed growth, reduce seedbank replenishment, and limit the spread of resistant populations.

This framework emphasizes the importance of timing, integration, and farmer coordination for sustainable weed control and resistance management in Mediterranean rice systems. Without systematic monitoring, diversification of herbicide modes of action, and implementation of integrated weed management, resistance levels are expected to continue expanding, posing a

substantial threat to the economic viability and sustainability of Greece's rice sector. Strategic interventions combining field, landscape, and policy-level actions are essential to maintain long-term productivity and prevent further escalation of herbicide-resistant weed populations.

7. Conclusion

Greek rice systems represent one of the most thoroughly documented herbicide resistance hotspots in Europe. Five major

weed species-*Echinochloa phyllopogon*, *E. oryzoides*, *E. crus-galli*, *E. colona*, *Cyperus difformis*, and weedy rice-exhibit confirmed resistance, including multiple and cross-resistance mechanisms, with *E. phyllopogon* being particularly problematic. ALS resistance is now widespread, frequently accompanied by failures in ACCase and auxinic herbicides. Glyphosate resistance is emerging in adjacent canal ecosystems, highlighting the importance of water-dispersed pathways. Resistance traits show minimal fitness costs, ensuring persistence across seasons and complicating control. Future of integrated weed management (IWM) in Greek rice systems will increasingly depend on the coordinated implementation of preventive, tactical, and long-term BMPs throughout the rice growth cycle.

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ΑΡΘΡΟ ΑΝΑΣΚΟΠΗΣΗΣ

Ζιζάνια ανθεκτικά στα ζιζανιοκτόνα στα Ελληνικά συστήματα καλλιέργειας ρυζιού: Ανασκόπηση της υφιστάμενης κατάστασης, των αιτιών και των επιπτώσεων στην Ολοκληρωμένη Διαχείριση

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Περίληψη Η ανθεκτικότητα στα ζιζανιοκτόνα αποτελεί σημαντικό περιοριστικό παράγοντα για τη βιώσιμη παραγωγή ρυζιού (*Oryza sativa* L.) στην Ελλάδα, όπου τα εντατικά συστήματα υδροσποράς βασίζονται κυρίως στη χημική καταπολέμηση ζιζανίων. Η ανασκόπηση συνθέτει τις υπάρχουσες γνώσεις για τα συστήματα παραγωγής ρυζιού, τα πρότυπα χρήσης ζιζανιοκτόνων και την εξέλιξη ανθεκτικών ζιζανίων στα ελληνικά αγροοικοσυστήματα. Τα κυρίαρχα ανθεκτικά είδη περιλαμβάνουν τα *Echinochloa* spp., *Cyperus difformis*, Weedy rice (red rice) και *Leptochloa fusca* subsp. *fascicularis*. Η ανθεκτικότητα έχει τεκμηριωθεί σε πολλαπλούς τρόπους δράσης, συμπεριλαμβανομένων αναστολέων ALS, ACCase, συνθετικών αυξινών και γλυφοσάτης, με αυξανόμενη εμφάνιση βιοτύπων με πολλαπλή ανθεκτικότητα. Η εξέλιξή της οφείλεται στη μακροχρόνια χρήση ζιζανιοκτόνων, στη μονοκαλλιέργεια και στη διασπορά μέσω δικτύων άρδευσης. Αξιολογούνται οι μηχανισμοί ανθεκτικότητας ανά είδος και προτείνονται στρατηγικές ολοκληρωμένης διαχείρισης ζιζανίων βασισμένες σε βέλτιστες πρακτικές διαχείρισης. Η εργασία αποτελεί αναφορά για ερευνητές, συμβούλους και υπεύθυνους πολιτικής και είναι εναρμονισμένη με τις αρχές Ολοκληρωμένης Φυτοπροστασίας της Ε.Ε.