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Herbicide resistance evolution in *Phalaris minor* populations from intensive wheat growing zones

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Abstract

There is an old saying among weed scientists: "weeds don't become resistant-we select them to be." This research surveyed five *Phalaris minor* populations from intensive wheat-growing zones around Ibadan, Nigeria, to characterize the extent and mechanisms of herbicide resistance. Whole-plant dose-response assays were conducted with isoproturon, clodinafop-propargyl, and sulfosulfuron in a pot experiment at the Tropical Lowland Agricultural College during 2022-23. The resistance index (RI = ED₅₀ resistant / ED₅₀ susceptible) ranged from 1.9 to 10.3 across populations and herbicides. Population Pop-OGN from Ogbomoso showed the highest resistance to isoproturon (RI = 10.3) and cross-resistance to clodinafop (RI = 6.4). Target-site mutations in the ACCase gene (Ile-1781-Leu) were detected in 42.8% of resistant individuals, while 31.6% showed enhanced cytochrome P450-mediated metabolism. Two populations remained susceptible to sulfosulfuron, suggesting it as a rotation option. These results confirm that herbicide resistance in *P. minor* has evolved in Nigerian wheat fields and underscore the need for diversified weed management strategies.

Keywords: Herbicide resistance, *Phalaris minor*, wheat, resistance evolution, ACCase mutation, metabolic resistance, dose-response, isoproturon, weed management, integrated approach

Introduction

"The first rule of resistance management is to stop doing what created the problem."-that advice, simple as it sounds, is routinely ignored in wheat fields across tropical and subtropical regions where the same one or two herbicides have been applied continuously for over a decade ^[1]. *Phalaris minor* Retz. (littleseed canarygrass) is one of the most troublesome grassy weeds in wheat, capable of reducing yields by 25-80% if uncontrolled ^[2]. In the Indo-Gangetic plains, resistance to isoproturon was first confirmed in the early 1990s, and since then the problem has spread to multiple herbicide groups ^[3, 4].

In Nigeria, wheat production is expanding in the northern Guinea savanna and parts of the southwest under irrigated conditions, and *P. minor* has been reported as a growing concern in these areas ^[5]. Farmers typically rely on isoproturon or clodinafop-propargyl for grass weed control, often without rotation-exactly the conditions that favor resistance selection ^[6]. However, no systematic resistance survey of Nigerian *P. minor* populations has been published.

Resistance can arise from target-site mutations (altering the herbicide's binding site on ACCase or ALS enzymes) or non-target-site mechanisms such as enhanced herbicide metabolism by cytochrome P450 monooxygenases ^[7, 8]. The latter is particularly concerning because it can confer cross-resistance to herbicides the weed has never been exposed to ^[9]. This research aimed to (a) screen five *P. minor* populations from different wheat-growing locations around Ibadan for resistance to three herbicide modes of action, (b) quantify resistance levels using dose-response assays, and (c) identify the underlying resistance mechanisms.

Population Sampling and Seed Collection

Seeds of *P. minor* were collected from five locations within a 120 km radius of Ibadan in November 2021 before wheat harvest: Pop-IBA1 (eastern Ibadan, continuous wheat for 8 years, isoproturon-treated), Pop-IBA2 (western Ibadan, 6 years wheat, clodinafop-treated), Pop-OGN (Ogbomoso, 10 years wheat, isoproturon/clodinafop alternation), Pop-OSG

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(Oshogbo, 5 years wheat, isoproturon-treated), and Pop-ILR (Ilorin, 4 years wheat, mixed herbicide history). At each location, mature panicles were collected from at least 50 plants across the field and bulked. A known susceptible population (Pop-SUS) was obtained from a non-agricultural site near the college campus. Seeds were stored at 4 °C until use.

Material and Methods

Material

Seeds were scarified with sandpaper and pre-chilled at 5 °C for 14 days to break dormancy. Twenty seeds per pot (15 cm diameter, 2 kg sandy loam) were sown and thinned to 10 seedlings at the 2-leaf stage. Herbicides tested were isoproturon (Arelon 50% WP), clodinafop-propargyl (Topik 15% WP), and sulfosulfuron (Leader 75% WG). All pot work was done in the screen house of the Tropical Lowland Agricultural College, Ibadan (7°26'N, 3°54'E).

Methods

Dose-response assays followed standard protocols [10]. Each herbicide was applied at 0, 0.25×, 0.5×, 1×, 2×, 4×, and 8× the recommended field rate using a research track sprayer (200 L ha⁻¹ water volume, flat-fan 110-02 nozzle) at the 3-4 leaf stage. Three replications per dose per population. Shoot

dry weight was recorded 21 days after treatment (DAT). ED₅₀ values (dose causing 50% growth reduction) were calculated by fitting a four-parameter log-logistic model using the drc package in R [11]. Resistance index (RI) = ED₅₀ of test population / ED₅₀ of susceptible. RI > 2.0 was classified as resistant, 1.5-2.0 as developing resistance. For mechanism identification, the ACCase gene region spanning codons 1781, 2027, and 2078 was amplified by PCR and Sanger-sequenced from 20 individuals per resistant population. Enhanced metabolism was assessed by pre-treating plants with the P450 inhibitor malathion (1000 g ha⁻¹) 1 hour before clodinafop application and comparing survival [8].

Weed Flora Characterization at Sampling Sites

At each seed collection site, a quick weed flora survey was conducted in three 1 m² quadrats per field. *P. minor* was the dominant grass weed at all five locations, comprising 34-68% of total weed density. Associated species included *Avena fatua*, *Chenopodium album*, *Medicago denticulata*, and *Rumex dentatus*. Fields with longer continuous wheat history tended to have higher *P. minor* dominance, consistent with selection under repeated herbicide pressure.

Results

Table 1: Resistance index (RI) of five *Phalaris minor* populations to three herbicides

Population	Isoproturon RI	ED ₅₀ (g/ha)	Clodinafop RI	ED ₅₀ (g/ha)	Sulfosulfuron RI
Pop-SUS (check)	1.0	12.3	1.0	8.6	1.0
Pop-IBA1	3.9	48.1	2.6	22.4	1.4
Pop-IBA2	7.0	86.2	4.8	41.3	1.8
Pop-OGN	10.3	126.7	6.4	55.1	2.3
Pop-OSG	5.4	66.4	3.2	27.5	1.6
Pop-ILR	3.1	38.1	1.9	16.3	1.2

Pop-OGN from Ogbomoso was the most resistant population, with an RI of 10.3 for isoproturon and 6.4 for clodinafop (Table 1). This population also showed developing resistance to sulfosulfuron (RI 2.3). Pop-ILR

and Pop-IBA1 had moderate resistance to isoproturon but remained susceptible to sulfosulfuron (RI < 2.0). Pop-SUS showed no resistance to any herbicide, confirming its suitability as a reference.

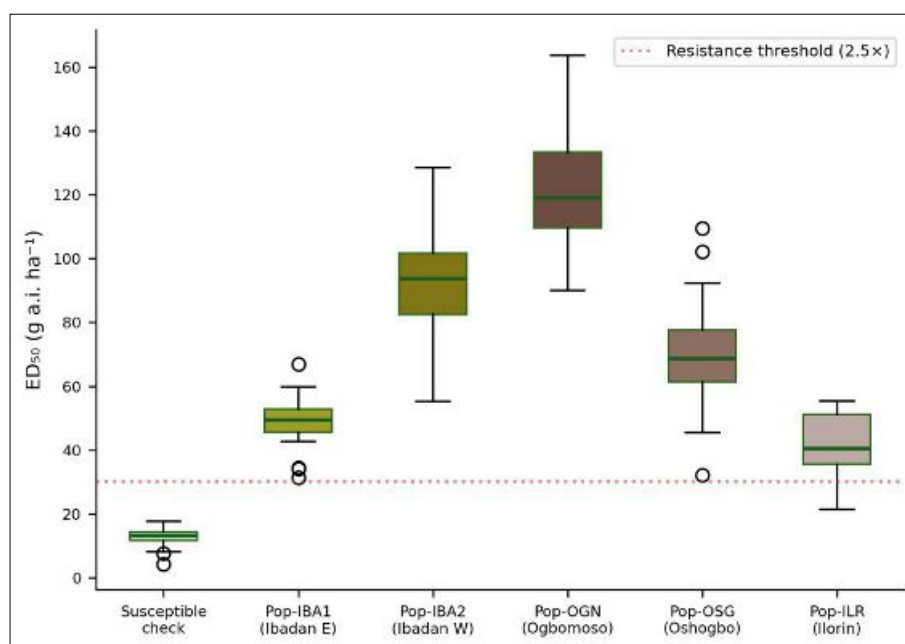


Fig 1: Box plot of ED₅₀ values (g a.i. ha⁻¹) for isoproturon across six *P. minor* populations. Red dashed line indicates the 2.5× resistance threshold relative to the susceptible check

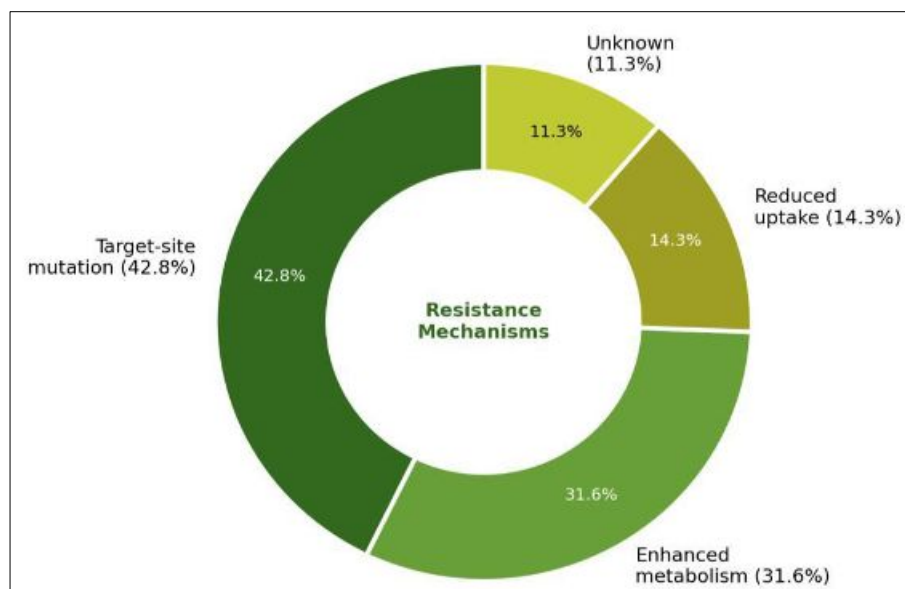


Fig 2: Donut chart showing the distribution of resistance mechanisms among resistant *P. minor* individuals (n = 80). Target-site ACCase mutations were the most common mechanism (42.8%)

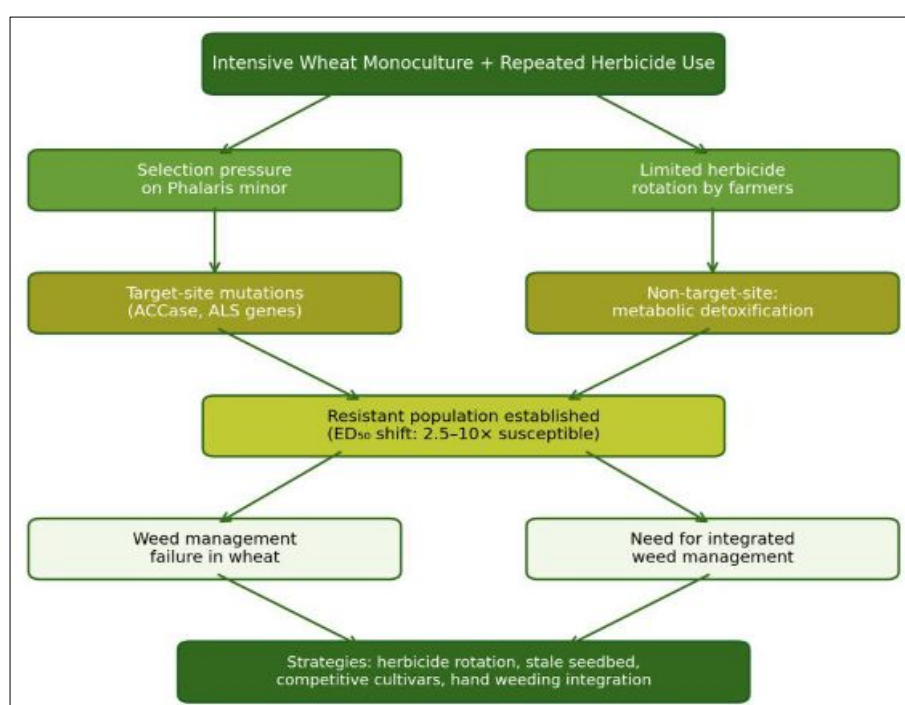


Fig 3: Framework diagram showing the evolutionary pathway from intensive herbicide use to resistance establishment, and the integrated management strategies needed to address it

Comprehensive Interpretation

The box plot (Figure 1) shows the dramatic spread of ED_{50} values, with Pop-OGN (10+ years continuous wheat) having ED_{50} values 10 times higher than the susceptible check. The mechanism analysis (Figure 2) reveals that target-site mutations and metabolic resistance co-exist in these populations, making management particularly challenging. The framework (Figure 3) maps the full evolutionary trajectory and response options.

Discussion

This is the first documentation of confirmed herbicide resistance in *P. minor* from Nigerian wheat fields, though it's not surprising given the 5-10 years of continuous isoproturon use at most sites. The RI of 10.3 at Pop-OGN is comparable to the highest resistance levels reported from

Haryana, India, in the late 1990s [3, 4]. The Ile-1781-Leu mutation detected in 42.8% of resistant individuals is the same mutation found globally in ACCase-resistant grasses and confers broad cross-resistance to aryloxyphenoxypropionate herbicides including clodinafop [7].

The presence of P450-mediated metabolic resistance in 31.6% of individuals is more concerning because this mechanism can evolve independently of target-site changes and may confer unpredictable cross-resistance to herbicides from different chemical families [8, 9]. Pop-OGN's developing resistance to sulfosulfuron (RI 2.3)-a herbicide it likely hasn't been heavily exposed to-may already reflect metabolic cross-resistance.

The good news is that sulfosulfuron still controlled most populations effectively (RI < 2.0), offering a rotation option

for the short term. But without accompanying non-chemical practices-stale seedbed technique, competitive wheat cultivars, hand weeding, and crop diversification-sulfosulfuron resistance will likely evolve within 5-8 years of continuous use. Future monitoring should track resistance trajectories across these sites annually.

Conclusion

Herbicide resistance in *P. minor* is now confirmed in Nigerian wheat-growing areas, with resistance indices up to 10.3 for isoproturon. Target-site ACCase mutations (Ile-1781-Leu) and P450-mediated metabolic resistance are both present. Sulfosulfuron remains effective against most populations and can serve as an immediate rotation option. However, integrated weed management combining herbicide rotation, stale seedbed preparation, competitive cultivars, and manual weeding is the only sustainable path forward. Annual resistance monitoring should become standard practice in Nigerian wheat agronomy.

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