agriculture

MDPI

Communication

Case Report of *Avena sterilis* subsp. *sterilis* ACCase Herbicide Resistance in Southern Spain

Carlos Sousa-Ortega [1], José Luis Fernandez [1] and Mino Sportelli [2,*]

[1] Departamento de Agronomía, Escuela Técnica Superior de Ingeniería Agrícola, Universidad de Sevilla, Ctr. Utrera Km 1, 41013 Seville, Spain
[2] Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy
* Correspondence: mino.sportelli@phd.unipi.it

Abstract: Wild oats are worldwide grassy weeds that cause substantial yield losses, particularly in winter cereal crops. In addition, wild oat herbicide resistant cases have increased; indeed, up to 52 cases have been registered. Despite this, no wild oat herbicide resistant cases have been described in Spain, where farmers and technicians have reported poor herbicide efficacy in sterile oats (*Avena sterilis* subsp. *sterilis* L.). A dose-response experiment was conducted comparing the behavior of two populations of *A. sterilis* from southern Spain to a susceptible population. These populations were collected from two commercial farms where a low efficacy of chemical control had been described. Clodinafop-propargyl and Pinoxaden were tested as active ingredients in the dose-response experiment. Additionally, an alternative herbicide, which consisted of a mixture of Mesosulfuron-methyl and Propoxycarbazone-Na, was also tested at a field dose. The two populations of *A. sterilis* studied provided a resistant factor higher than 10 for Clodinafop-propargyl and higher than 4 for Pinoxaden. A total control was achieved for plants treated with Mesosulfuron-methyl and Propoxycarbazone-Na.

Keywords: wild oat; *Avena sterilis*; herbicide resistance; acetyl-CoA carboxylase; Clodinafop-propargyl; Pinoxaden

Citation: Sousa-Ortega, C.; Fernandez, J.L.; Sportelli, M. Case Report of *Avena sterilis* subsp. *sterilis* ACCase Herbicide Resistance in Southern Spain. *Agriculture* **2023**, *13*, 85. https://doi.org/10.3390/agriculture13010085

Academic Editors: Anna Wenda-Piesik and Agnieszka Synowiec

Received: 2 December 2022
Revised: 23 December 2022
Accepted: 27 December 2022
Published: 28 December 2022

1. Introduction

Wild oats are a troublesome grassy weeds that can be found in many agricultural areas [1]. These plants are a major problem in the Mediterranean basin, because they infest winter cereal fields, sometimes taking over more than 80% of the field by harvest time [2]. Due to their competitive behavior, these weeds pose a threat to cereal crops, and can reduce their yields by up to 60% [3]. The aggressive nature of these weeds is exploited by a large production of seeds with an early shedding and a consistent dormancy [3,4]. Wild oats have a great ability to significantly increase their presence in the soil seedbank. According to Gonzalez-Andujar and Bastida [5], if control is not provided, the wild oats seedbank can increase by 10 times in two growing seasons. Wild oats consist of two main species: spring wild oat (*Avena fatua* L.) and winter wild oat (*A. sterilis*). The latter species is composed by two accepted subspecies called *A. sterilis* subsp. *ludoviciana* (Durieu) Gillet & Magne) and *A. sterilis* subsp. *sterilis* [6]. *A. sterilis* subsp. *sterilis* is a weed that is widely distributed throughout Spain, particularly in the warmer regions of the center and south [7]. This weed causes substantial losses to cereal crops [8]. This species is mostly found in cereal fields in patches of irregular shapes [9]. The effective control of sterile oat in winter cereal crops commonly relies on the application of selective post-emergence herbicides that inhibit the activity of acetyl-CoA carboxylase (ACCase, mode of action group 1). This herbicide family affects fatty acid biosynthesis by the carboxylation of acetyl-CoA to malonyl-CoA. It is recommended that these active ingredients should be applied in conjunction with a safener in order to reduce the possibility of crop damage and to achieve the desired selectivity between weeds and cash crops [10]. The large-scale application of herbicides from the

ACCase family has resulted in 262 unique cases of herbicide resistance in 50 weed species. There are a total of 52 unique herbicide resistance cases that have been registered in wild oats species, with 38, 10, and 4 cases in *A. fatua*, *A. sterilis* subsp. *sterilis*, and *A. sterilis* subsp. *ludoviciana*, respectively [11]. According to Papapanagiotou et al. [12], most of these cases have developed target site resistance. Target site resistance is supplied by gene mutations in target enzymes (i.e., acetyl-CoA carboxylase (ACCase), and so on) and has been extensively investigated in herbicide resistant weed species [13]. Conversely, non-target-site resistance mechanisms have been reported as decreased herbicide penetration or translocation in the plant, with the aim of reducing the herbicide concentration reaching the target-site [13]. Non-target-site resistance also includes mechanisms that promote the degradation of herbicide molecules by plant metabolism (hence, commonly called metabolic resistance) [13]. The aim of this study was to assess the herbicide resistance to Clodinafop-propargyl and Pinoxaden of two *A. sterilis* subsp. *sterilis* biotypes. This is significant because, despite the numerous recorded instances of herbicide resistance among wild oats species, there have been no reports of herbicide-resistant *Avena sterilis* subsp. *sterilis* in Spain [11]. However, poor herbicide efficacy in sterile oat is a problem that technicians and farmers in southern Spain are facing.

2. Materials and Methods

2.1. Preliminary Experiment

In autumn of 2021, a preliminary trial was carried out over two different populations of *A. sterilis* subsp. *stetrilis* (hereafter *A. sterilis*). Each population (hereafter bio 1 and bio 2) originated from two different commercial farms placed in Ronda (lat = 36°45′ N, long = 5°09′ W, Málaga, southern Spain), in which herbicide application showed poor efficacy during previous seasons. In particular, farmers from this area have been reporting this issue since 2019. For this reason, a total of 60 seedlings (30 from bio 1 and 30 from bio 2) were collected and transplanted to 10 cm × 10 cm and 10.5 cm deep plastic pots. Two active ingredients providing ACCase inhibition (acetyl-CoA carboxylase, group 1) were applied separately to different pots. Clodinafop-propargyl was applied at 0, 24, 48, 72, and 96 g of a. i. ha^{-1} rates, while Pinoxaden was applied at 0, 15, 30, 45, and 60 g of a. i. ha^{-1} rates. The selected rates of active ingredient represented for both products the 0, 0.5, 1, 1.5 and 2 times the field recommended doses. The plants were treated at the tillering stage with the main shoot plus three-five tillers in the Higher Technical School of Agricultural Engineering of the University of Seville (ETSIA, lat = 37°21′ N, long = 5°56′ W). Herbicides were sprayed by means of a precision bench sprayer delivering 300 L ha^{-1} at a pressure of 2.2 Bar. This preliminary trial showed that all plants treated at maximum doses of both active ingredients survived. Additionally, a susceptible population collected from Jerez in (lat = 36°41′ N, lon = 6°08′ W, southern Spain) was tested at the same dosage. All individuals of this population died at the 0.5 field dose. Since these data did not show intermedium levels of mortality, a non-linear regression could not be performed.

2.2. Plant Material

Seeds from the two mentioned populations of *A. sterilis* were collected from more than 20 individuals in June 2022. The susceptible population used in this dose-response experiment came from an organic farm in Jerez (lat = 36°41′ N, lon = 6°08′ W, southern Spain) and were collected in 2015. After collection, the seeds were cleaned and dry-stored at 4 °C in a refrigerator until the experiments started.

2.3. Experimental Design

The experiment was conducted at ETSIA. Initially, seeds of each population were sown in plastic seedbeds with 3 cm of diameter and 4 cm of depth on 15 December 2022. Seedbeds were placed in a growing chamber with an average daytime temperature of 24 ± 1 °C, a relative humidity of 60%, and supplementary lighting providing a PPFD of 1000−1200 μmol m^{-2} s^{-1} at the top of the pot with a 12 h photoperiod (08:00–20:00 h).

After seeds germination, seedlings were transplanted to 10 cm × 10 cm and 10.5 cm deep pots and were brought outside in an open field. Pots were filled with a 50% mixture of a peat moss (Gramoflor, Gramoflor GmbH& Co.Kg, Neuenkirchen-Vörden, Germany) and silica sand (Sisanflor, Sisanflor S.L, Sisante, Cuenca, Spain). All plants were irrigated with tap water to avoid drought stress. A total of six repetitions (pots) were included for each dose in a completely randomized block design. On 3 March 2022, with plants at the three-five tillers stage, herbicides were sprayed by means of a precision bench sprayer delivering 300 L ha^{-1} at a pressure of 2.2 Bar. The treatment day presented adequate climatic conditions with a temperature of 17 °C without wind. As in the preliminary experiment, two active ingredients providing ACC inhibition were tested separately. Details of the applied herbicide treatments are reported in Table 1.

Table 1. Details of the herbicide treatments applied in the dose-response experimental tests.

Site of Action	Herbicide	Commercial Name	Supplier	Test Doses (g a.i. ha^{-1})	
				Preliminary Trial	Dose-Response Trial
ACCase	Clodinafop-propargyl	Topik® 240 EC	Syngenta, Basel, Switzerland	0, 24, 48, 72, 96	0, 12, 24, 48, 72, 96, 192
	Pinoxaden	Axial® Pro	Syngenta, Basel, Switzerland	0, 15, 30, 45, 60	0, 7.5, 15, 30, 45, 60, 120
ALS	Mesosulfuron-methyl + Propoxycarbazone-Na	Monolith®	Bayer AG, Leverkusen, Germany	-	14.99 (Mesosulfuron-methyl) + 22.48 (Propoxycarbazone-Na)

ACCase, acetyl-CoA carboxylase; ALS, acetolactate synthase; The field recommended rate is 48 g a.i. ha^{-1} for Clodinafop-propargyl and 30 g a.i. ha^{-1} for Pinoxaden.

An adjuvant (Adigor®, Syngenta, Basel, Switzerland) was added to Clodinafop-propargyl as it is recommended (0.5%), while it was not necessary to add adjuvant to Pinoxaden treatments, since it was already present in the product's formulation. Both herbicide formulations included cloquintocet-mexyl as a safener, which was founding at 5.55% *w*/*w* (weight/weight) in TOPIK® 240 EC (Clodinafop-propargyl) and 1.55% *w*/*w* in the Axial® Pro (Pinoxaden). Herbicide application rates are reported in Table 1, and in both cases represent the 0, 0.5, 1, 1.5, 2 and 4 times the field recommended doses.

Additionally, an alternative herbicide, which inhibits acetolactate synthase (ALS, group 2), was tested. This additional treatment consisted of a mixture of Mesosulfuron-methyl and Propoxycarbazone-Na (MONOLITH®, Bayer AG, Leverkusen, Germany). This herbicide formulation also included the mefenpir-dietil safener, which was present at 9%. Moreover, this herbicide was applied with the adjuvant Biopower® (Bayer AG, Leverkusen, Germany), as it is recommended (0.5%). An application rate at the field recommended dose was applied for this treatment (Table 1).

Finally, weed control efficacy in terms of mortality (%) from each dose was evaluated for all the treatments at 28 days after treatment. The average temperature between the herbicide application and the final evaluation was 13.5 °C, and achieved 22.4 °C and 5.3 °C as maximum and minimum temperatures.

2.4. Statistical Analysis

Dose-response data was analyzed using the 'drc' package [14] from R software (Version 4.2.1; R Core team, Vienna, Austria) [15]. The non-linear regression used to fit was a logistic equation with four parameters (Equation (1)).

$$Y = c + \left(\frac{d - c}{1 + e^{b\ [\log(X) - \log(e)]}} \right) \quad (1)$$

where c is the lower limit, d is the upper limit, e is the herbicide rate required for 50% mortality (LD_{50}), and b is the slope at LD_{50} [14]. In this equation, the Y and X variables represent the percentage of mortality and the herbicide dose.

Finally, the resistance factor (RF) was calculated as the division of the resistant population's LD_{50} by the susceptible population's LD_{50} (Equation (2)).

$$RF = \frac{Resistant\ population\ LD_{50}}{Susceptible\ population\ LD_{50}} \quad (2)$$

3. Results

In the preliminary experiment, all individuals from bio 1 and bio 2 survived at twice the field recommended dose, while all susceptible individuals died at half of the field recommended dose. These results implied that the RF of the bio 1 and bio 2 population was greater than four for Clodinafop-propargyl and Pinoxaden.

The bio 2 population presented a resistance to Clodinafop-propargyl treatment that was 11.4 times higher than the susceptible population in terms of mortality. The resistance factor of the population bio 1 could not be evaluated, since no plants of this population died at any doses of Clodinafop-propargyl. Only the susceptible population was fully controlled at the field dose. Indeed, the LD_{50} and LD_{90} estimated for the susceptible population was 78% and 71% lower than the field dose, respectively. In contrast, the LD_{50} and LD_{90} for the bio 2 population was 66% and 85% higher, respectively, than the field dose (Table 2 and Figure 1). When plants were treated with Pinoxaden, bio 1 and bio 2 populations presented a resistant factor that was 6.8 and 4.6 times higher than the susceptible population in terms of mortality. Only the susceptible population was fully controlled at the field dose. Furthermore, the LD_{50} and LD_{90} estimated for this population was 80% and 71% lower than the field dose, respectively. On the other hand, both bio 1 and bio 2 populations obtained a LD_{50} value that was 50% higher than the field dose. The LD_{90} instead, was 59% and 46% higher than the field doses for bio 1 and bio 2 populations, respectively (Table 2 and Figure 1). The alternative herbicide formulation (Mesosulfuron-methyl and Propoxycarbazone-Na) tested at the field doses achieved 100% mortality for the three populations.

Table 2. Values of herbicide rate required to achieve the 50% (LD_{50}) and 90% (LD_{90}) mortality for each herbicide and population. The resistant factor (RF) is also presented for both parameters.

Herbicide	Biotype	Regresion Parameters		LD_{50}		LD_{90}	
		b	c	Value	RF	Value	RF
Clodinafop-propargyl	Susceptible	7.742	0.050	10.4	-	13.8	-
	bio 2	2.742	4.633	118.9	11.4	264.9	19.2
Pinoxaden	Susceptible	8.670	0.073	6.6	-	8.5	-
	bio 1	31.926	29.995	44.6	6.8	47.8	5.6
	bio 2	6.039	22.034	30.5	4.6	43.9	5.2

The RF of the population bio 1 for Clodinafop -propargyl could not be calculated, since no plants of this population died. The value c is the lower limit, and b is the slope at LD_{50} obtained from the four parameters logistic regression. The parameter d (the upper limit) was 100 in all the non-linear regressions and, for this reason, is not reported on the table.

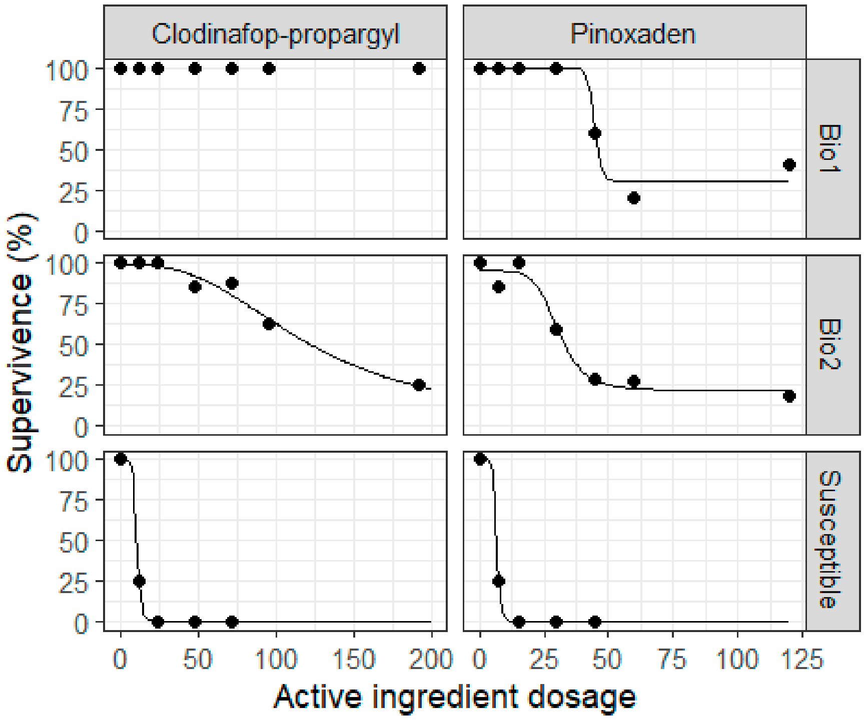

Figure 1. Dose-response of each population to both active ingredient doses. The black points represent the observed results and the lines indicate the logistic regression for each situation.

4. Discussion

The trial sought to understand the levels of herbicide resistance in two populations of sterile oats. Studied populations provided a resistant factor of 10 for Clodinafop-propargyl and 4 for Pinoxaden. A total control was achieved for plants treated with Mesosulfuron-methyl and Propoxycarbazone-Na. According to Cruz-Hipolito et al. [16], a population was considered resistant when the resistant factor was higher than two. The behavior of the two populations in this study was similar for Pinoxaden, while an unclear situation was found for Clodinafop-propargyl, with bio 1 showing a higher resistance than bio 2. Several cases of wild oats ACCase herbicide resistance have been found in the previous literature. For instance, Papapanagiotou et al. [17] found different sterile oat populations that were resistant to Clodinafop-propargyl and Pinoxaden in Greece. Wild oat ACCase family herbicide resistant populations were studied in Chile and Mexico by Cruz-Hipolito et al. [16]. In those cases, a resistant factor ranging from 2.2 to 16.4 for different herbicides was obtained. The authors provided evidence that the resistance was attributed to metabolic mechanisms. The current study suggests that the herbicide resistance in the two populations in southern Spain may have been caused by a target-site mutation, as populations only showed resistances to the same herbicide family [18], and some individuals survived at increased dosage applications [19]. However, additional fields and lab trials are required to validate this statement. In southern Spain, grass weeds from cereal fields are typically controlled by the ACCase or ALS herbicide family. These findings suggest that the only herbicide capable of selectively controlling the studied biotypes would be herbicides from the ALS family, as both biotypes exhibited herbicide resistance to the two main active ingredients derived from the ACCase family. However, the use of these ALS inhibitor herbicides is particularly vulnerable to gene point mutations that easily confer resistance. In fact, the ALS herbicides family has been linked to more herbicide resistant cases than any other herbicide group [18].

Author Contributions: Conceptualization, C.S.-O. and J.L.F.; methodology, C.S.-O. and J.L.F.; software, C.S.-O.; validation, C.S.-O. and J.L.F.; formal analysis, C.S.-O.; investigation, C.S.-O., M.S. and J.L.F.; resources, J.L.F.; data curation, C.S.-O. and J.L.F.; writing—original draft preparation, M.S. and C.S.-O.; writing—review and editing, M.S.; visualization, M.S. and C.S.-O.; supervision, C.S.-O.; project administration, M.S.; funding acquisition, M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank the Cátedra ADAMA for providing the materials necessary to carry out the herbicide applications.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Holm, L.; Doll, J.; Holm, E.; Pancho, J.V.; Herberger, J.P. *World Weeds: Natural Histories and Distribution*; John Wiley & Sons: Hoboken, NJ, USA, 1997; ISBN 978-0-471-04701-8.
2. Travlos, I.; Giannopolitis, C.N.; Paspatis, E.A. Wild Oat Variability in Wheat Fields of Viotia in Central Greece. *Hell. Plant Prot. J.* **2008**, *1*, 107–112.
3. Bajwa, A.A.; Akhter, M.J.; Iqbal, N.; Peerzada, A.M.; Hanif, Z.; Manalil, S.; Hashim, S.; Ali, H.H.; Kebaso, L.; Frimpong, D.; et al. Biology and Management of *Avena fatua* and *Avena ludoviciana*: Two Noxious Weed Species of Agro-Ecosystems. *Environ. Sci. Pollut. Res.* **2017**, *24*, 19465–19479. [CrossRef] [PubMed]
4. Barroso, J.; Navarrete, L.; Sanchez Del Arco, M.J.; Fernandez-Quintanilla, C.; Lutman, P.J.W.; Perry, N.H.; Hull, R.I. Dispersal of *Avena fatua* and *Avena sterilis* Patches by Natural Dissemination, Soil Tillage and Combine Harvesters. *Weed Res.* **2006**, *46*, 118–128. [CrossRef]
5. Gonzalez-Andujar, J.L.; Bastida, F. Modeling the Population Dynamics of a Community of Two Grass Weeds of Winter Wheat in a Mediterranean Area. *Int. J. Plant Prod.* **2018**, *12*, 219–223. [CrossRef]
6. Sousa-Ortega, C.; Royo-Esnal, A.; Loureiro, I.; Marí, A.I.; Lezáun, J.A.; Cordero, F.; Saavedra, M.; Paramio, J.A.; Fernández, J.L.; Torra, J.; et al. Modeling Emergence of Sterile Oat (*Avena sterilis* ssp. *ludoviciana) under Semiarid Conditions. Weed Sci.* **2021**, *69*, 341–352. [CrossRef]
7. García-Baudín, J.M. Importancia de las avenas locas en España. *Bol. Serv. Plagas* **1982**, *8*, 35–42.
8. Gonzalez-Andujar, J.L.; Fernandez-Quintanilla, C.; Bastida, F.; Calvo, R.; Gonzalez-Diaz, L.; Izquierdo, J.; Lezaun, J.A.; Perea, F.; Sanchez Del Arco, M.J.; Urbano, J.M. Field Evaluation of a Decision Support System for Herbicidal Control of *Avena sterilis* ssp. *ludoviciana in Winter Wheat. Weed Res.* **2010**, *50*, 83–88. [CrossRef]
9. Ruiz, D.; Escribano, C.; Fernández-Quintanilla, C. Assessing the Opportunity for Site-Specific Management of *Avena sterilis* in Winter Barley Fields in Spain. *Weed Res.* **2006**, *46*, 379–387. [CrossRef]
10. Délye, C.; Zhang, X.-Q.; Michel, S.; Matéjicek, A.; Powles, S.B. Molecular Bases for Sensitivity to Acetyl-Coenzyme A Carboxylase Inhibitors in Black-Grass. *Plant Physiol.* **2005**, *137*, 794–806. [CrossRef] [PubMed]
11. Heap, I. The International Survey of Herbicide Resistant Weeds. Available online: http://www.weedscience.com (accessed on 14 October 2022).
12. Papapanagiotou, A.P.; Damalas, C.A.; Menexes, G.C.; Eleftherohorinos, I.G. Resistance Levels and Chemical Control Options of Sterile Oat (*Avena sterilis* L.) in Northern Greece. *Int. J. Pest Manag.* **2020**, *66*, 106–115. [CrossRef]
13. Fernández-Moreno, P.T.; Alcantara-de la Cruz, R.; Cruz-Hipólito, H.E.; Rojano-Delgado, A.M.; Travlos, I.; De Prado, R. Non-Target Site Tolerance Mechanisms Describe Tolerance to Glyphosate in *Avena sterilis*. *Front. Plant Sci.* **2016**, *7*, 1220. [CrossRef] [PubMed]
14. Ritz, C.; Baty, F.; Streibig, J.C.; Gerhard, D. Dose-Response Analysis Using R. *PLoS ONE* **2015**, *10*, e0146021. [CrossRef] [PubMed]
15. R Development Core Team. *R: A Language and Environment for Statistical Computing*; Team, R.D.C., Ed.; R Foundation for Statistical Computing: Vienna, Austria, 2017; Volume 1, ISBN 3-900051-07-0.
16. Cruz-Hipolito, H.; Osuna, M.D.; Domínguez-Valenzuela, J.A.; Espinoza, N.; De Prado, R. Mechanism of Resistance to ACCase-Inhibiting Herbicides in Wild Oat (*Avena fatua*) from Latin America. *J. Agric. Food Chem.* **2011**, *59*, 7261–7267. [CrossRef] [PubMed]
17. Papapanagiotou, A.P.; Kaloumenos, N.S.; Eleftherohorinos, I.G. Sterile Oat (*Avena sterilis* L.) Cross-Resistance Profile to ACCase-Inhibiting Herbicides in Greece. *Crop Prot.* **2012**, *35*, 118–126. [CrossRef]

18. Heap, I. Global Perspective of Herbicide-Resistant Weeds. *Pest Manag. Sci.* **2014**, *70*, 1306–1315. [CrossRef] [PubMed]
19. Alebrahim, M.T.; Zangoueinejad, R.; Tseng, T.M. Biochemical and Molecular Knowledge about Developing Herbicide-Resistant Weeds. In *Herbicide Resistance in Weeds and Crops*; Intech Open: London, UK, 2017; pp. 101–132, ISBN 978-953-51-3556-2.