AN ABSTRACT OF THE THESIS OF

Lucas Kopecky Bobadilla for the degree of Master of Science in Crop Science presented on May 29, 2019

Title: Frequency, Distribution, Ploidy Diversity and Control Options of Herbicide Resistant Italian Ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) Populations in Western Oregon

Abstract approved:

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Italian ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) is one of the most troublesome weeds with respect to herbicide resistance selection. Some reasons for this are the numerous documented cases of multiple and cross herbicide resistance and some of the biological characteristics of this species, such as wind cross-pollination that allows the rapid spread of some resistance traits. Oregon can be considered a unique place to study Italian ryegrass resistance management due to high production of grass seed crops in the western part of the state. The dominant grass seeds crop species grown in western Oregon are Italian/perennial ryegrass and tall fescue (*Schedonorus arundinaceus (*Schreb.*)* Dumort.). Both crops require a high purity in the final seed lots making the management of other grass weeds vital. Seeds carrying herbicide resistance into a seed lot must be avoided.

Oregon Italian ryegrass production is divided into two types of cultivars: diploids (2x = 2n = 14) and tetraploids (2x = 4n = 28); conversely, all weedy biotypes of

Italian ryegrass documented as a weed species are diploid. No studies have documented the ploidy diversity in the Willamette Valley area. A survey was conducted to quantify the frequency, distribution, ploidy diversity and herbicide resistance in populations of Italian ryegrass in the Willamette Valley. A total of 150 fields were surveyed between 2017 and 2018. Fifty percent (75 fields) of the fields had Italian ryegrass present with the majority of those located in the northern surveyed area. In these fields, 42% (32 populations) had high Italian ryegrass density levels (20 or more plants/$m^2$). Herbicide screening tests were conducted for 11 herbicides: clethodim, pinoxaden, quizalofop-p-ethyl, glyphosate, glufosinate, paraquat, mesosulfuron, pyroxsulam, pronamide, flufenacet + metribuzin, and pyroxasulfone. For the screened populations, 88% (66 populations) were classified as having presence of resistance to at least one herbicide tested. Around 6% (5 populations) of the tested Italian ryegrass populations were tetraploids. No resistance traits were confirmed in tetraploid populations.

A combination of both multiple and cross-resistance with a frequency of 61% (46 populations) was identified in the tested populations. Plant density level was correlated with the presence of multiple resistance (considering populations with high presence of resistance level). The odds-ratio of finding cross-resistance, considering only high presence of resistance level, was higher by a factor of 3.12 in wheat fields when compared to tall fescue fields. The most frequent resistance was to the following modes of action: ACCase, ALS and EPSPs inhibitors. According to herbicide screening tests, glufosinate and pyroxasulfone are still options to control Italian ryegrass but some cases of glufosinate resistance were already documented in

Oregon. Cluster patterns of multiple resistance with were identified in the surveyed area. This research was the first survey of spatial distribution, frequency of herbicide resistance and ploidy diversity of Italian ryegrass in western Oregon. These numerous cases of resistance found in western Oregon creates a need for new management approaches.

Research on rangeland areas showed that synthetic auxin herbicides can affect seed viability and can be used as a management tool to reduce the seed production of invasive annual grasses. However, no studies have been conducted on the effect to Italian ryegrass and the feasibility as a management practice in tall fescue grown for seed. Greenhouse and field trials were conducted to quantify the effects of synthetic auxin herbicides on the seed viability of Italian ryegrass and tall fescue. Two years of greenhouse trials were conducted in 2017 and 2018. Four field trials were conducted in western Oregon between 2017 and 2018. Eight synthetic auxins herbicide treatments were tested:  two rates of 2,4-D, two rates of dicamba, aminopyralid, 2,4-D + dicamba, 2,4-D + clopyralid and halauxyfen-methyl. Results indicate that aminopyralid reduced seed viability of different biotypes of Italian ryegrass both in controlled and field environments. Aminopyralid reduced the viability of Italian ryegrass seeds; however, tall fescue was more sensitive to this treatment making this management method not applicable for this crop. Aminopyralid still might be used for other cropping systems and understanding the mechanism involved on the seed viability reduction could elucidate better ways to use this management practice. Thus, future studies should explore the physiological mechanism involved in this effect,

quantify the recommend rates of aminopyralid and determine if other crops might be tolerant to the application of aminopyralid.

# Frequency, Distribution, Ploidy Diversity and Control Options of Herbicide Resistant Italian Ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) Populations in Western Oregon

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Lucas Kopecky Bobadilla, Author

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# CONTRIBUTION OF AUTHORS

Dr. Andrew G. Hulting proposed and advised all steps of this research. He was involved in all stages of the preparation of this research thesis and providing feedbacks until its conclusion. Dr. Carol Mallory-Smith provided help with feedbacks, ideas during the conduction of this research project. She was also involved in the improvement of the chapters. Dr. Marcelo L. Moretti provided ideas and feedback during all the stages of this research. Dr. Ryan Contreras was involved in providing access to laboratory equipment and teaching how to conduct essential steps for the conclusion of Chapter 2 properly. Pete A. Berry was actively involved in the spatial analysis and map production in Chapter 2. Dan Curtis provided help and assistance in all field trials conducted in Chapter 3.

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**FREQUENCY, DISTRIBUTION AND CONTROL OPTIONS OF HERBICIDE RESISTANT ITALIAN RYEGRASS *Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) POPULATIONS IN WESTERN OREGON.**

# CHAPTER 1: General Introduction

## Biology and Taxonomy of Italian Ryegrass

Italian ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) is a cool season species in the *Poaceae* family which typically has annual life cycle but sometimes can be either a biennial or a short-lived perennial. Significant growth occurs in the winter and fall in Oregon (UC IPM (http://tiny.cc/wgan6y)). It is an introduced species originally from temperate regions of Europe which now is ubitiquous in the USA.

Italian ryegrass is an obligate out-crossing species with a self-incompatibility of almost 98%.[1,2] Like most grasses, this species reproduces by seeds. Italian ryegrass can produce a large number of small seeds formed on an inflorescence in a spike (USDA plants (http://tiny.cc/6nan6y). Italian ryegrass is wind pollinated and its pollen may travel long distances. Previous studies have shown that 99% of the crosses occurred in a range of 8.6 m from a 1 $m^2$ pollen source.[3,4] Plants have two-sided spikes with spikelets laterally flattered, 10 to 22 florets per spikelet, leaves are rolled in bud and lemmas with awns that can reach 15 mm. This species can grow to 150 cm in height.[5]

Due to high frequencies of hybridization of ryegrass species with other grasses from the *Lolium* and *Festuca* genera, the ploidy level can vary from one population of Italian ryegrass to another.[6,7] This species is commonly referred to as diploid (2x = 14).[8]

## Herbicide Resistance in Italian Ryegrass

With respect to numbers of individuals, biomass, area covered and diversity of habitat where found, grasses are one of the most successful plant families in the world.[5] The characteristics that have contributed to this success have also enabled many species to become aggressive invaders of natural and agricultural ecosystems.[9] The structural design of grasses helps make them highly competitive with crop species.[10] The presence of axillary buds at the base of each internode allows most grasses to resprout when damaged.[10] In addition to these factors that make grasses good competitors for nutrients in an agricultural environment, some grasses have evolved resistant populations to many herbicides and different modes of action.[11]

Herbicide resistance is defined as an evolutionary phenomenon whereby happens through intensive use of herbicides that generate a selective pressure for certain individuals carrying resistance traits in a plant population.[12] Genetics plays a significant role in the regulation of the frequency, number, dominance and fitness cost of the resistance.[12] Another important factor is related to the biology of the plant that encompasses the type of reproduction, seed production and longevity of seed in the soil seed bank. The herbicide characteristics, the frequency of application also play a role in the selection of herbicide resistant weeds.[12] In some cases the selection of herbicide resistant populations can occur due to the use of sublethal doses, a phenomenon not exclusive in plants but also seen in bacteria, fungi and insects.[13–16]

There are 500 unique cases of herbicide resistance are already documented worldwide in 256 species (Heap I. (www.weedscience.org)). The most common modes of action with reported resistance cases are the acetolactate synthase (ALS) inhibitor herbicides

followed by photosystem II inhibitors (PSII), acetyl CoA Carboxylase (ACCase) inhibitors, and EPSPs (5-enolpyruvylshikimate-3-phosphate synthase) inhibitor herbicides, respectively. Italian ryegrass has documented unique resistant cases in eight different modes of action (Heap I. (www.weedscience.org).

Multiple-resistance (resistance to more than one herbicide from different modes of action) and cross-resistance (resistance to herbicide from different chemical families but the same mode of action) cases are common in Italian ryegrass populations worldwide.[17] The fact that no new herbicide mode of action has been released in the last 30 years has increased the number of cases of multiple resistance due to lack of alternative chemical control options.[18,19]

Previous research indicates that Italian ryegrass has a predisposition to evolve and select populations that carry herbicide resistance traits.[20–24] This predisposition can be attributed to the species being an obligated outcrossing and have a large seed production resulting into a high genetic variation within populations.[13,22,25,26] Oregon has 16 unique herbicide resistance cases documented within these, four cases are in Italian ryegrass (Heap I. (www.weedscience.org).

## Gene Flow and Ploidy Level

Gene flow and genetic drift can be an important part in the evolution and introgression of traits in a species. Previous research shows that the differences that could not be explained within a single population were most often explained as a selected adaption provided by gene drift from outside the population. Gene flow with hybridization is known to be an essential part of the evolutionary theory among

populations and species in delivering novel adaptive variations to specific populations.[27–29]

Gene flow can be defined as the spread and introduction of a genetic trait and alleles from a population to another.[28,30] Gene flow is a well-recognized component of evolution in plants and has played important roles in many cases both in domestic plants as well as in natural adaptation and invasiveness.

Gene flow occurred long before plant domestication as in the case of the hybridization of many plants species like durum wheat, bread wheat, and maize.[30–32] Gene flow between domesticated and natural populations via either pollen or seed contamination; this can occur within the same species or between species and is commonly referred to as introgression.[33]

Introgression can be defined as the introduction of genetic material from one individual into another due to backcrossing of the formed hybrid with the parent species; [34,35] however, the success of introgression is dependent of the recovery of fertility. Thus, crosses between weeds and crops, even with different ploidy levels, can occur under natural field conditions.[31,36] An example in Oregon of crop/weed gene flow, occurs between glyphosate-resistant creeping bentgrass (*Agrostis stolonifera*) to susceptible bentgrass and wild relatives in Oregon.[37] Another case is the hybridization of *Salsola australis* and *Sausola tragus* in California resulting in a viable allohexaploid tumbleweed hybrid.[38]

Most of the documented gene flow between crop and weeds occurs from the crop to the wild relative; however, there are some cases and possibilities where weeds are passing traits to crops or exchanging them among other weed populations.[39]

Due to considerable diversity of grass species grown in Oregon and the large number of troublesome grass weeds, hybridization between weeds and cultivated grasses could occur. Hybridization between wheat (*Triticum aestivum*) and jointed goatgrass (*Aegilops cylindrica*) was demonstrated previously in Oregon.[40]

Italian ryegrass is known as a species that can hybridize with other species.[27] Cases of natural hybrids with the *Festuca* species such as meadow fescue (*Festuca pratensis*) have been documented.[41] The genetic similarity between *Festuca* and *Lolium* genera was previously documented showing that the allohexaploid tall fescue (*Schedonorus arundinaceus (*Schreb*.)* Dumort.) arises due to the cross affinity of ryegrass species and meadow fescue.[42] This relationship has been used in breeding programs for the creation of a hybrid known as *Festulolium.*[6,43] The major concern about hybridization between these grasses species is related to the possibility of movement of herbicide resistance traits between weedy Italian ryegrass biotypes and related species.

Despite the risk of hybridization with other grass species, another issue is related to the gene flow among wild biotypes and planted cultivars. Italian ryegrass is the dominant grass seed crop cultivated in Oregon as well as one of the major weeds in most grass seed crops in the state (OSU grass and legume extension (http://tiny.cc/n9in6y). Because they are the same species, crosses between them could occur and gene flow of alleles that provide herbicide resistance could be passed to the crop. This scenario was occured in *Brassica* species.[44]

Studies regarding pollen clouds could be a next step to understand the presence of different ryegrass with different ploidy levels.[24,45,46] Previous research has shown that it is possible to identify differences on ploidy levels in pollen due to size differences between

a 2n and an n pollen grain of perennial ryegrass.[47] However, other studies showed that these two ploidy levels can still be compatible and can produce viable seeds but with a unequal segregation. Other observation were low vigor, poor development and greatly reduced germination rates with a large number of triploids produced on the crosses of diploid perennial ryegrass with different ploidy levels.[8,48]

## Weed Survey

Weeds have shown a vast diversity of behavior in reproduction, dispersion, and adaptation to different situations and environments. One possible first step to understand these characteristics is to conduct a weed survey to help growers to understand these parameters.

Weed surveys can have a different focus depending on the objectives of it. Examples are to understand weed diversity,[49,50] characterizing weed species,[51] risks of hybridization,[52] and distribution of herbicide-resistant cases.[53] Weed surveys can also be used to confirm field trials or previous results of characterizing weed management problems.[49,54,55]

Surveys can serve as supplementary information to field experiments due to the substantial possibility of spatial related factors variation such as weather, soil and elevation that could lead to possible inconsistent trial results.[49] This type of observational survey can provide valuable data to answer questions or serve as the first step for answer more complex questions related to weed management and ecology.[56]

In the western region of the United States, surveys have been conducted to understand specific patterns of herbicide resistance of some weed species. A survey was conducted in the Central Valley of California to assess the spread of glyphosate-resistant horseweed

(*Conyza canadensis)* in perennial crop production system.[57] Another study had the objective to understand the spread of glyphosate resistance in Italian ryegrass in northern California in a broad diversity of crops and non-crop areas.[58] Oregon still lack information provided by a weed survey that could help to answer complex questions and supplement results field trials to achieve a better understanding of some weed patterns and characteristics.

## Synthetic Auxin Herbicides

Synthetic auxin herbicides are classified as herbicides specifically for the control broadleaf plants and were introduced into the agricultural market place in the 1940s.[59] These types of herbicides are classified by the HRAC (Herbicide Resistance Action Committee) as group O (HRAC (http://tiny.cc/enjn6y) and by the WSSA (Weed Science Society of America) as group 4 (WSSA (http://tiny.cc/bpjn6y). Synthetic auxin herbicides are extensively used, with approximately 366 x $10^6$ hectares treated worldwide.[59]

The first herbicide of this group to be commercialized was 2,4-D, and its use has not declined in the last 70 years of use.[60] New synthetic auxin molecule discoveries have been occurring more frequently when compared to other modes of action. The discovery (Figure 1.1) of new molecules started with picloram in 1963 which led to the discovery of aminopyralid in 2006[61] with the formulation of the molecule halauxifen-methyl and florpyrauxifen-benzyl more recently synthesized.[62,63] Synthetic auxin herbicides are classified into seven subclasses (Figure 1.2): (a) phenoxy-carboxylic acids; (b) benzoic acids; (c) pyridine carboxylic acid; (d) quinoline carboxylic acid; (e) arylpicolinic Acid; (f) pyridyloxy-carboxylic acids and, (g) pyrimidine-carboxylic acids.[59,64,65]

These herbicides mimic the plant hormone auxin also known as indole-3-acetic acid (IAA). To understand how these herbicides work, it is necessary to understand the function of IAA. Plant metabolism, growth and other responses to biotic and abiotic factors are controlled by phytohormones.[66,67] Auxins are specific hormones predominantly synthesized in new leaves and found most in areas of the plant.[68]

The numerous auxin target sites can explain the importance of auxin to plants. The hormone can affect embryo development, root development, shoot development, stress responses, leaf development, and stomata establishment. Twenty-three types of IAA are known to exist in plants as well as three classes of auxin receptors: (1) Auxin binding protein1 (ABP1); (2) S-phase kinase-associated protein 2A (SKP2A); (3) TIR1 (Transporter inhibitor response) and AFB1-5 (Auxin signaling F-Box).[69]

The mechanism of action and how synthetic auxin herbicides lead to plant death still unclear due to some gaps of research.[60] Plants are likely killed by an "auxin overdose"; initially the cells of the plant start to respond to this massive amount of auxin at the plasma membrane when the auxin first binds to the auxin-receptor protein ABP1. This activation alters the cytoskeleton leading to epinasty and alters the movement of peroxisomes and mitochondria making the cells vulnerable to ROS (reactive oxygen species) effects.[70] Another process occurs with the synthetic auxin acting at the Ubiquinatin route which activates the TIR1/AFB1-5 proteins that will activate the genes responsible for the production of ethylene and ABA (abscisic acid) synthesis in larger quantities that they naturally occur leading to a production of ROS, creating an oxidative environment in the cells. The sum of these effects cause the unsaturation of lipids on cell membranes followed by cell leakage and death.[64,69,71,72]

These effects are usually observed in dicot plants. However, some grasses are controlled by the use of synthetic auxin herbicides such as quinclorac. Examples of species that can be affected by quinclorac are *Echinocloa, Digitaria* and *Braquiaria* species.[73] The biosynthesis of ABA, ethylene, and production of ROS was shown to not lead to death in grasses.[64,73,74] Previous research showed that quinclorac induces ethylene production that will induce cyanide compound production which is thought to be the mechanism for plant death in this case.[75] Another more recently discovered synthetic auxin herbicide, pyridine-2-carboxylate, used in rice can control some grasses and sedges as well.[62]

Despite these known effects of quinclorac and other synthetic auxin herbicide to grasses, some other effects were also observed in previous studies. Herbicides such as picloram, clopyralid, dicamba, and aminopyralid can affect seed viability and fecundity of some grasses such as wheat and corn when applied at a specific growth stages.[76–81] The exact physiological and molecular explanation for these effects are still unknown. However, previous research already has shown that some auxin receptors genes are highly linked with seed size and viability formation as well as in essential steps of ovule formation in *Arabidopsis* flowers.[82] Thus, more studies are required to determine the feasibility of these effects as a management practice for grass weed control.

## Research Objectives

Italian ryegrass is considered one of the most troublesome weeds worldwide. However, this specific weed represents a unique problem in Oregon due to the majority production of the area are focused on grass seed production. This species can cause problems in Oregon due to herbicide resistance and gene flow between related crops and

weed. Resistance is reducing the number of chemical tools available to control Italian ryegrass creating the demand and necessity of new approaches for management. There is also a need to understand the spatial context of Italian ryegrass herbicide resistance in Oregon as an initial step to understand and design the best approach for management. The following research was conducted as a starting point for a new approach to control and understand the distribution and frequency of herbicide resistance in Italian ryegrass in western Oregon. A survey and greenhouse/field trials were conducted to address these needs and to test possible new management approaches using synthetic auxin herbicides.

## References

1. Fearon C, Hayward M, Lawrence M. Self-incompatibility in ryegrass. *Heredity*. **50**:35–45 (1983).

2. Karn E, Jasieniuk M. Genetic diversity and structure of *Lolium perenne* ssp. *multiflorum* in California vineyards and orchards indicate potential for spread of herbicide resistance via gene flow. *Evol Appl*. **10**:616–29 (2017).

3. Giddings G. Modelling the spread of pollen from *Lolium perenne*. The implications for the release of wind-pollinated transgenics. *Theor Appl Genet*. **100**:971–4 (2000).

4. Giddings G, Hamilton NS, Hayward M. The release of genetically modified grasses. Part 1: pollen dispersal to traps in *Lolium perenne*. *Theor Appl Genet*. **94**:1000–6 (1997).

5. Meyers SC. Flora of Oregon. Volume 1, Pteridophytes, gymnosperms, and monocots. 1st ed. Vol. 1. Fort Worth, Texas: Botanical Research institute; pp. 591 (2015).

6. Kopecký D, Loureiro J, Zwierzykowski Z, Ghesquière M, Doležel J. Genome constitution and evolution in *Lolium* × *Festuca* hybrid cultivars (*Festulolium*). *Theor Appl Genet*. **113**:731–42 (2006).

7. Lewis E. *Festuca* L. x *Lolium* L.= *Festulolium* Aschers and Grabn. *Stace CA Ed Hybrid Xora Br Isles*.547–52 (1975).

8. Lamote V, Baert J, Roldán-Ruiz I, De Loose M, Van Bockstaele E. Tracing of 2n egg occurrence in perennial ryegrass (*Lolium perenne* L.) using interploidy crosses. *Euphytica*. **123**:159–64 (2002).

9. Pyšek P, Skálová H, Čuda J, Guo W-Y, Suda J, Doležal J, et al. Small genome separates native and invasive populations in an ecologically important cosmopolitan grass. *Ecology*. **99**:79–90 (2018).

10. Gibbs Russell G, Watson L, Koekemoer M, Smook L, Barker N, Anderson H, et al. Grasses of southern Africa. Memoirs of the botanical survey of South Africa. 2nd ed. Vol. 58. National Botanic Gardens, Botanical Research Institute; pp. 437 (1990).

11. Bock DG, Kantar MB, Caseys C, Matthey-Doret R, Rieseberg LH. Evolution of invasiveness by genetic accommodation. *Nat Ecol Evol*. **2**:991 (2018).

12. Powles SB, Yu Q. Evolution in action: plants resistant to herbicides. *Annu Rev Plant Biol*. **61**:317–47 (2010).

13. Busi R, Powles SB. Evolution of glyphosate resistance in a *Lolium rigidum* population by glyphosate selection at sublethal doses. *Heredity*. **103**:318–25 (2009).

14. Olofsson SK, Cars O. Optimizing drug exposure to minimize selection of antibiotic resistance. *Clin Infect Dis*. **45**:129–36 (2007).

15. Roush RT, McKenzie JA. Ecological genetics of insecticide and acaricide resistance. *Annu Rev Entomol*. **32**:361–80 (1987).

16. Shaw M. Is there such a thing as a fungicide resistance strategy? A modeller's perspective. *Asp Appl Biol*. **78**:37 (2006).

17. Brunharo CA, Hanson BD. Multiple herbicide–resistant Italian ryegrass [*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot] in California perennial crops: characterization, mechanism of resistance, and chemical management. *Weed Sci*. **66**:696–701 (2018).

18. Délye C, Jasieniuk M, Le Corre V. Deciphering the evolution of herbicide resistance in weeds. *Trends Genet*. **29**:649–58 (2013).

19. Duke SO. Why have no new herbicide modes of action appeared in recent years? *Pest Manag Sci*. **68**:505–12 (2012).

20. Avila-Garcia WV, Mallory-Smith C. Glyphosate-resistant Italian ryegrass (*Lolium perenne*) populations also exhibit resistance to glufosinate. *Weed Sci*. **59**:305–309 (2011).

21. Llewellyn RS, Powles SB. High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheat belt of Western Australia. *Weed Technol*. **15**:242–248 (2001).

22. Bararpour MT, Norsworthy JK, Burgos NR, Korres NE, Gbur EE. Identification and biological characteristics of ryegrass (*Lolium* spp.) accessions in Arkansas. *Weed Sci*. **65**:350–60 (2017).

23. Betts KJ, Ehlke NJ, Wyse DL, Gronwald JW, Somers DA. Mechanism of inheritance of diclofop resistance in Italian ryegrass (*Lolium multiflorum*). *Weed Sci*. **40**:184–189 (1992).

24. Owen MJ, Martinez NJ, Powles SB. Multiple herbicide-resistant *Lolium rigidum* (annual ryegrass) now dominates across the Western Australian grain belt. *Weed Res*. **54**:314–324 (2014).

25. Karn E, Beffa R, Jasieniuk M. Variation in response and resistance to glyphosate and glufosinate in California populations of Italian ryegrass (*Lolium perenne* ssp. *multiflorum*). *Weed Sci*. **66**:168–79 (2018).

26. Powles SB, Lorraine-Colwill DF, Dellow JJ, Preston C. Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed Sci*. **46**:604–7 (1998).

27. Ellstrand NC. Current knowledge of gene flow in plants: implications for transgene flow. *Philos Trans Biol Sci*. **358**:1163–70 (2003).

28. Levin DA, Kerster HW. Gene flow in seed plants. In: *Gene flow in seed plants*. Springer; p.139–220 (1974).

29. Stebbins GL. Variation and evolution in plants: progress during the past twenty years. In: *Essays in evolution and genetics in honor of Theodosius Dobzhansky*. Boston, MA: Springer; p.173–208 (1970).

30. Ellstrand NC, Rieseberg LH. When gene flow really matters: gene flow in applied evolutionary biology. *Evol Appl*. **9**:833–6 (2016).

31. Dewitt N. Gene flow from crops to weeds. *Nat Biotechnol*. **17**:318 (1999).

32. Feldman M, Sears ER. The wild gene resources of wheat. *Sci Am*. **244**:102–13 (1981).

33. Martins BAB, Leonard JM, Sun L, Zemetra RS, Mallory-Smith C. Selection pressure effects on the proportion and movement of resistance alleles introgressed from wheat in *Aegilops cylindrica*. *Weed Res*. **56**:293–303 (2016).

34. Ellstrand NC, Prentice HC, Hancock JF. Gene flow and introgression from domesticated plants into their wild relatives. *Annu Rev Ecol Syst*. **30**:539–63 (1999).

35. Henderson IR, Salt DE. Natural genetic variation and hybridization in plants. *J Exp Bot*. **68**:5415 (2017).

36. Warwick SI. Gene flow between GM crops and related species in Canada. *First Decade Herbic Resist Crops Can Top Can Weed Sci*. **4**:101–13 (2007).

37. Zapiola M, Campbell C, Butler M, Mallory-Smith C. Escape and establishment of transgenic glyphosate-resistant creeping bentgrass (*Agrostis stolonifera*) in Oregon, USA: a 4-year study. *J Appl Ecol*. **45**:486–94 (2008).

38. Welles SR, Ellstrand NC. Genetic structure reveals a history of multiple independent origins followed by admixture in the allopolyploid weed *Salsola ryanii*. *Evol Appl*. **9**:871–8 (2016).

39. Sarangi D, Tyre AJ, Patterson EL, Gaines TA, Irmak S, Knezevic SZ, et al. Pollen-mediated gene flow from glyphosate-resistant common waterhemp (*Amaranthus rudis* Sauer): consequences for the dispersal of resistance genes. *Sci Rep*. **7**:44913 (2017).

40. Martins BAB, Leonard JM, Sun L, Zemetra RS, Mallory-Smith C. Selection pressure effects on the proportion and movement of resistance alleles introgressed from wheat in *Aegilops cylindrica*. *Weed Res*. **56**:293–303 (2016).

41. Wit F. Natural and experimental hybrids of ryegrasses and meadow fescue. *Euphytica*. **13**:294–304 (1964).

42. Pašakinskienė I, Anamthawat-Jonsson K, Humphreys M, Paplauskiene V, Jones R. New molecular evidence on genome relationships and chromosome identification in fescue (*Festuca*) and ryegrass (*Lolium*). *Heredity*. **81**:659 (1998).

43. Akiyama Y, Ueyama Y, Hamada S, Kubota A, Kato D, Yamada-Akiyama H, et al. Utilization of flow cytometry for *festulolium* breeding (*Lolium multiflorum* (2x)× *Festuca arundinacea* (6x)). *Breed Sci*. **66**:234–43 (2016).

44. Hüsken A, Dietz-Pfeilstetter A. Pollen-mediated intraspecific gene flow from herbicide resistant oilseed rape (*Brassica napus* L.). *Transgenic Res*. **16**:557–69 (2007).

45. Millwood R, Nageswara-Rao M, Ye R, Terry-Emert E, Johnson CR, Hanson M, et al. Pollen-mediated gene flow from transgenic to non-transgenic switchgrass (*Panicum virgatum* L.) in the field. *BMC Biotechnol*. **17**:40 (2017).

46. Ganie ZA, Jhala AJ. Modeling pollen-mediated gene flow from glyphosate-resistant to-susceptible giant ragweed (*Ambrosia trifida* L.) under field conditions. *Sci Rep*. **7**:17067 (2017).

47. Jansen RC, Den Nijs APM. A statistical mixture model for estimating the proportion of unreduced pollen grains in perennial ryegrass (*Lolium perenne* L.) via the size of pollen grains. *Euphytica*. **70**:205–215 (1993).

48. Griffiths DJ, Pegler RAD, Tonguthaisri T. Cross compatibility between diploid and tetraploid perennial ryegrass (*Lolium perenne* L.). *Euphytica*. **20**:102–12 (1971).

49. Hanzlik K, Gerowitt B. Methods to conduct and analyze weed surveys in arable farming: a review. *Agron Sustain Dev*. **36**:11 (2016).

50. Leeson J, Sheard J, Thomas A. Weed communities associated with arable Saskatchewan farm management systems. *Can J Plant Sci*. **80**:177–85 (2000).

51. Fried G, Norton LR, Reboud X. Environmental and management factors determining weed species composition and diversity in France. *Agric Ecosyst Environ*. **128**:68–76 (2008).

52. Ohadi S, Littlejohn M, Mesgaran M, Rooney W, Bagavathiannan M. Surveying the spatial distribution of feral sorghum (*Sorghum bicolor* L.) and its sympatry with johnsongrass (*S. halepense*) in South Texas. *PLOS ONE*. **13**:e0195511 (2018).

53. Owen MJ, Powles SB. Distribution and frequency of herbicide-resistant wild oat (*Avena* spp.) across the Western Australian grain belt. *Crop Pasture Sci*. **60**:25–31 (2009).

54. Fried G, Chauvel B, Reboud X. A functional analysis of large-scale temporal shifts from 1970 to 2000 in weed assemblages of sunflower crops in France. *J Veg Sci*. **20**:49–58 (2009).

55. Lutman P, Storkey J, Martin H, Holland J. Abundance of weeds in arable fields in southern England in 2007/08. *Asp Appl Biol*. **91**:163–8 (2009).

56. Leeson J, Sheard J, Thomas A. Multivariate classification of farming systems for use in integrated pest management studies. *Can J Plant Sci*. **79**:647–54 (1999).

57. Hanson BD, Shrestha A, Shaner DL. Distribution of glyphosate-resistant horseweed (*Conyza canadensis*) and relationship to cropping systems in the Central Valley of California. *Weed Sci*. **57**:48–53 (2009).

58. Jasieniuk M, Ahmad R, Sherwood AM, Firestone JL, Perez-Jones A, Lanini WT, et al. Glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in California: distribution, response to glyphosate, and molecular evidence for an altered target enzyme. *Weed Sci*. **56**:496–502 (2008).

59. Busi R, Goggin DE, Heap IM, Horak MJ, Jugulam M, Masters RA, et al. Weed resistance to synthetic auxin herbicides. *Pest Manag Sci*. **74**:2265–76 (2018).

60. Peterson MA, McMaster SA, Riechers DE, Skelton J, Stahlman PW. 2,4-D past, present, and future: a review. *Weed Technol*. **30**:303–45 (2016).

61. Masters RA, Burch PL, Brueninger J, Carrithers VF, Jachetta J, Kline WN, et al. Aminopyralid: a new herbicide for pasture vegetation management. In: Proceedings of the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America International Annual Meeting; 2005 Nov 10. Salt Lake City, Utah; (2005).

62. Epp JB, Alexander AL, Balko TW, Buysse AM, Brewster WK, Bryan K, et al. The discovery of Arylex™ active and Rinskor™ active: Two novel auxin herbicides. *Bioorg Med Chem*. **24**:362–71 (2016).

63. Epp JB, Schmitzer PR, Crouse GD. Fifty years of herbicide research: comparing the discovery of trifluralin and halauxifen-methyl. *Pest Manag Sci*. **74**:9–16 (2018).

64. Christoffoleti PJ, Figueiredo MRA de, Peres LEP, Nissen S, Gaines T, Christoffoleti PJ, et al. Auxinic herbicides, mechanisms of action, and weed resistance: A look into recent plant science advances. *Sci Agric*. **72**:356–62 (2015).

65. Devine M, Duke SO, Fedtke C. Physiology of herbicide action. 1st ed. Englewood Cliffs, New Jersey: PTR Prentice Hall; pp. 441 (1992).

66. Fahad S, Hussain S, Matloob A, Khan FA, Khaliq A, Saud S, et al. Phytohormones and plant responses to salinity stress: a review. *Plant Growth Regul*. **75**:391–404 (2015).

67. Wani SH, Kumar V, Shriram V, Sah SK. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *Crop J*. **4**:162–76 (2016).

68. Flasiński M, Hąc-Wydro K. Natural vs synthetic auxin: Studies on the interactions between plant hormones and biological membrane lipids. *Environ Res*. **133**:123–34 (2014).

69. Salehin M, Bagchi R, Estelle M. SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *Plant Cell*. **27**:9–19 (2015).

70. Rodríguez-Serrano M, Pazmiño DM, Sparkes I, Rochetti A, Hawes C, Romero-Puertas MC, et al. 2,4-Dichlorophenoxyacetic acid promotes S-nitrosylation and oxidation of actin affecting cytoskeleton and peroxisomal dynamics. *J Exp Bot*. **65**:4783–93 (2014).

71. Badescu GO, Napier RM. Receptors for auxin: will it all end in TIRs? *Trends Plant Sci*. **11**:217–23 (2006).

72. Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, Estelle M, et al. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature*. **446**:640–5 (2007).

73. Grossmann K. Auxin herbicides: current status of mechanism and mode of action. *Pest Manag Sci*. **66**:113–20 (2010).

74. Grossmann K. Mediation of herbicide effects by hormone interactions. *J Plant Growth Regul*. **22**:109–22 (2003).

75. Gao Y, Li J, Pan X, Liu D, Napier R, Dong L. Quinclorac resistance induced by the suppression of the expression of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase genes in *Echinochloa crus-galli* var. *zelayensis*. *Pestic Biochem Physiol*. **146**:25–32 (2018).

76. Andersson L. Effects of MCPA and tribenuron-methyl on seed production and seed size of annual weeds. *Swed J Agric Res*. **24**:49–56 (1994).

77. Ball DA. Effects of aminocyclopyrachlor herbicide on downy brome (*Bromus tectorum*) seed production under field conditions. *Invasive Plant Sci Manag*. **7**:561–4 (2014).

78. Crone EE, Marler M, Pearson DE. Non-target effects of broadleaf herbicide on a native perennial forb: a demographic framework for assessing and minimizing impacts. *J Appl Ecol*. **46**:673–82 (2009).

79. Rinella MJ, Masters RA, Bellows SE. Growth regulator herbicides prevent invasive annual grass seed production under field conditions. *Rangel Ecol Manag*. **63**:487–490 (2010).

80. Rinella MJ, Haferkamp MR, Masters RA, Muscha JM, Bellows SE, Vermeire LT. Growth regulator herbicides prevent invasive annual grass seed production. *Invasive Plant Sci Manag*. **3**:12–6 (2010).

81. Rinella MJ, Masters RA, Bellows SE. Effects of growth regulator herbicide on downy brome (*Bromus tectorum*) seed production. *Invasive Plant Sci Manag*. **6**:60–4 (2013).

82. Schruff MC, Spielman M, Tiwari S, Adams S, Fenby N, Scott RJ. The AUXIN RESPONSE FACTOR 2 gene of *Arabidopsis* links auxin signalling, cell division, and the size of seeds and other organs. *Development*. **133**:251–61 (2006).

# Figures

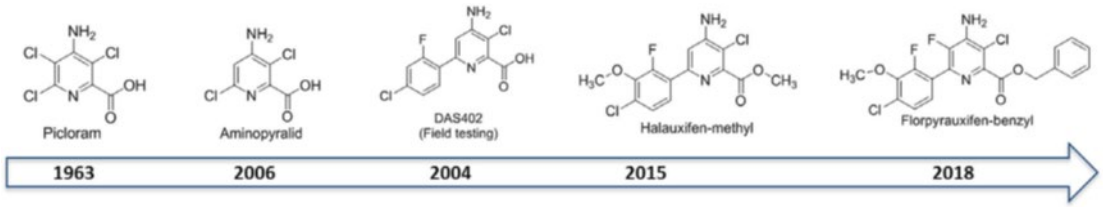



Figure 1-1 Herbicide discovery timeline of some synthetic auxin herbicides

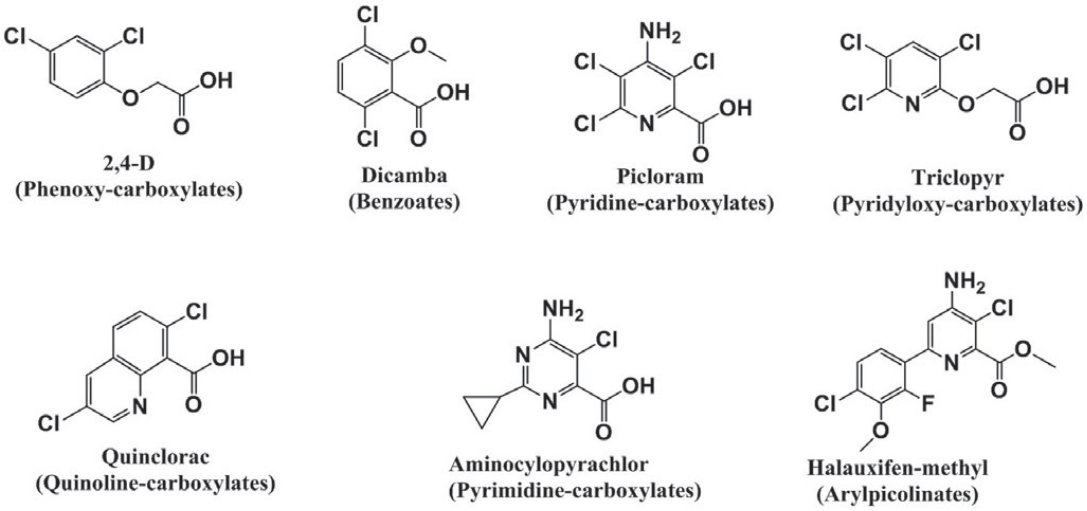



Figure 1-2 Major molecules of each subgroup of synthetic auxin herbicides

# CHAPTER 2: Frequency, distribution and ploidy diversity of herbicide-resistant Italian ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) populations in the Willamette Valley of western Oregon

## ABSTRACT

**BACKGROUND**: Italian ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) is one of the most troublesome weeds worldwide. A major grass seed crop grown in Oregon is Italian ryegrass as well as one of the major weeds, creating a scenario where crosses between weed and crop and, seed contamination are likely. Oregon grows two types of Italian ryegrass cultivars: diploid and tetraploid. No cases of tetraploid plants have been documented as being weedy or carrying herbicide resistance. A survey was conducted to understand the distribution, frequency and ploidy levels of herbicide resistance to some to the 11 most often used herbicides to control Italian ryegrass populations in western Oregon.

**RESULTS:** At total 150 fields were surveyed where 50% (75) had Italian ryegrass present. Herbicide resistant populations were documented in 88% (66) of the tested populations. Resistance to ALS, ACCase and EPSP herbicides were the most frequent types of resistance. Multiple resistance was found in 75% (55) of the tested populations and a spatial cluster pattern of populations was found. Most cases of resistance were found in the northern part of the surveyed area (26 populations). A correlation between plant density and multiple resistance presence was found. Most cases of ALS and ACCase resistance were found in wheat fields. Tetraploid populations of Italian ryegrass were found but no cases of herbicide resistance were documented. Based on herbicide screening studies, pyroxasulfone is still an option to manage Italian ryegrass.

**CONCLUSION:** To our knowledge, this is the first survey on herbicide resistance and ploidy diversity in Italian ryegrass in western Oregon. Cases of high presence of resistance were equally distributed across the surveyed area indicating that this is a general problem and not to specific locations. The herbicides glufosinate and pyroxasulfone still provided control options, however, if used as the only management tool, can create a high selection pressure for the evolution of resistant biotypes. This survey will serve as a basis for future studies regarding understanding the spread and evolution of resistance and how to minimize it. Other surveys should be conducted in the following years to create a multiple year database to allow further analysis to check the evolution of herbicide resistance in the long term.

## Introduction

Weed management is one of the greatest challenges of any agricultural system and over time has become even more difficult. With the exponential growth of the human population worldwide and the constantly expanding demands for a more efficient agriculture, weed management will become even more important. [1] These demands have culminated into an intensive overuse of herbicides that selected herbicide resistance traits in weed populations.[2] More than 500 unique cases of herbicide resistance have been documented worldwide within 256 species (Heap I. (www.weedscience.org). Italian ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) is one of the most troublesome weeds worldwide with resistance to eight different sites of action already documented (Heap I. (www.weedscience.org). Traits including obligate outcrossing and a large amount of seed production, make Italian ryegrass an excellent candidate species for evolution of resistance traits due to a high genetic variability. [2–4]

The state of Oregon, USA, is considered to be the major grass seed production area in the world (USDA 2017 census of agriculture (www.nass.usda.gov/AgCensus/) which makes Oregon a unique place to study Italian ryegrass weed management. The major grass seed species grown in Oregon are Italian ryegrass, perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Schedonorus arundinaceus (*Schreb*.)* Dumort.) (Anderson N. (http://tiny.cc/n9in6y). These crops require a high seed purity in the final product making the management of weeds vital. Another important factor in Italian ryegrass management in Oregon is the fact that Italian ryegrass is cultivated as a crop and is also one of the major weeds in the state making them fully compatible for crosses. Research has shown that crosses between weeds and crop may occur, which could be a bridge for introduction of herbicide resistance into the crop. [5–9]

Oregon Italian ryegrass production includes two types of cultivars: diploids (2n = 2x = 14) (Kew RBG (http://data.kew.org/sid/) and tetraploids (2n = 4x = 28) [10]; No studies have been conducted to document the ploidy diversity of Italian ryegrass to confirm if only diploid populations can be weedy or to determine the invasiveness potential of tetraploid cultivars. No studies had shown if the presence of herbicide resistance is exclusive to diploid populations of Italian ryegrass.

Understanding the distribution and the frequency of herbicide resistance in a region, is a first step to gather information for farmers, agronomists and the industry about the best approaches to minimize it. [11–17] Despite the well documented cases of herbicide resistance of Italian ryegrass in Oregon, [9,18–20] a survey has not been conducted to understand the overall scenario of herbicide resistance in the Willamette Valley.

The objective of this study was to measure the frequency and distribution of Italian ryegrass and resistance to common herbicides used in grass seed and wheat production and to assess the ploidy levels of Italian ryegrass populations in Oregon.

# Material and Methods

## Survey and sampling design

A 2-year survey was conducted in 2017 and 2018 in the Willamette Valley in Oregon (Figure 2.1) to gather information about the frequency, distribution and ploidy level of herbicide resistant Italian ryegrass in grasses grown for seed and wheat fields. This survey was conducted using a stratified design where the Willamette Valley was divided into three strata (North, Center and South) according to agricultural land acreage of the crops of interest (Figure 2.2). The surveyed sites were randomly selected using available data from USDA - CropScape regarding crop geospatial location [21] to generate location data of grass seed crops and wheat fields in the Willamette Valley. Random selection of fields was made using a fishnet of rectangular cells in ArcMap (ISRI 2019 (http://www.arcgis.com), the center of each cell was used to create a point and those that fell inside a field of at least 8 hectares where then filtered and 225 fields where randomly selected for each year. From these fields, a total of 75 randomly selected fields from the initial randomization for each year were included in the survey due to reasons such as no access to the fields or precision issues. The focus of the survey was on wheat and tall fescue; however, CropScape data were not precise enough to differentiate between grass species and thus many of the randomized points were grass species other than tall fescue or other types of crops. These points in other crops were surveyed, but if the field

contained either Italian ryegrass or perennial ryegrass as a crop (98 fields in 2017 and 105 fields in 2018), it was not included in the survey.

Sampling each year was conducted during the summer close to grass seed maturity (months of June and July) to ensure that Italian ryegrass was present, and seeds could be harvested. A visual estimate of the populations was made in a 60 $m^2$ in each field by walking in a "W" formation. Italian ryegrass plant density in the 60 $m^2$ area was visually estimated. Variables collected in each area were crop type, Italian ryegrass presence and density, elevation and GPS coordinates. Italian ryegrass density was evaluated as three levels: high (20 plants or more per square meter), medium (10 to 19 plants per square meter) and low (1 to 9 plants per square meter). Crop type was recorded as wheat, tall fescue, clover, orchardgrass, or tree crops (hazelnuts and plums). Presence of Italian ryegrass was evaluated as present or absent. If Italian ryegrass was present, seeds of plants present were collected and brought to the laboratory for further analysis for herbicide resistance and ploidy level determination.

## Herbicide resistance screening

### *Whole plant screening*

Whole plant screening for resistance to post-emergence herbicides was conducted in a greenhouse trial using a complete randomized design with four replications. One hundred seeds of each population were separated into four 11x11x2.8 cm germination boxes (156C container, Hoffman Manufacturing Inc; Corvallis, OR 97330 USA) with 20 ml of distilled water in blue blotter paper and germinated in growth chambers set for 12 h of light at 23/15 ºC. After 10 days, 16 seedlings were transplanted equally distant from each

other into four 24 by 24 cm square plastic trays filled with commercial potting mix (Sunshine mix 1 Potting mix, Sun Gro Horticulture, Inc., 110th Ave. NE, Suite 490, Bellevue, WA 98004). Each tray was used as a replication. Plants were watered daily and treated once a week with a standard 20-20-20 fertilizer (Miracle-Gro water soluble; The Scotts Company LLC, Maryville Ohio, USA). Herbicides were applied when plants reached the two-leaf stage and were 10 to 16 cm in height. Herbicides were applied using an air cabinet sprayer (Generation III Spray Chamber, De Vries Manufacturing, 86956 State HWY 251, Hollandale, MN 56054) with a flat-fan spray *Teejet nozzle* 8004 set to deliver 187 L $ha^{-1}$. Survival count and image analysis to measure green area reduction using ImageJ software [22] were collected 21 days after treatment. For the image analysis, trays were placed over a black background and a Fujifilm Xpro-2 with a 23 mm 2.0 Fujifilm lens was set into a tripod with the lens at 64 cm from the soil surface; a photo from a ruler placed in the soil surface of a tray was taken to calibrate the scale of the software. Camera was set to capture raw files with 4,000 x 6,000-pixel resolution and images were converted to tiff files. The color threshold method ISOdata [23] using a Lab color space [24] with a coloring space of *L* ranging between 80 – 255, *a* ranging from 0 – 132 and *b* ranging from 132 – 255 was utilized to measure the green area of each file. To analyze multiple photos at once, a macro was made to loop the analysis over a folder with the images (codes available in Appendix A).

Populations were classified as having one of three resistance levels to each herbicide tested: high presence of resistance (20% or more of survival and less than 70% of green area reduction), low presence of resistance (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction

of at least 90%). Populations were also classified according to the type of resistance: Multiple-resistance was considered present if the population presented high or low presence of resistance to more than one herbicide from different mode of action while cross resistance was considered present if the population presented high or low presence of resistance to more than one herbicide from different chemical families but same mode of action.

***Seed bioassay screening***

Resistance screening to multiple pre-emergence herbicides was conducted using a laboratory bioassay to measure seed germination in a medium containing a discriminant herbicide rate (Table 2.2). This methodology was used with the objective to isolate the plant response to only the herbicide and to facilitate germination counts and, speed of data collection. This assay was developed based on previous research using this method to detect resistant grass weed species biotypes to Acetyl CoA Carboxylase (ACCase) inhibitors. [25–27]

Herbicide rates were defined by conducting a seed bioassay dose response using a four-parameter log-logistic dose response (Table A.2). For each herbicide, three known susceptible populations ('Gulf', 'Tetraploid' and 'DAN') were used to define the rate that would control all the susceptible populations. For the flufenacet + metribuzin dose response treatment (Figure A.1), two known resistant populations ('DK' and 'PR') were used as a positive control. For the pyroxasulfone (Figure A.2) and pronamide (Figure A.3) treatments only two susceptible biotypes were used due to the fact that no resistant populations were available. Seeds were placed in 9 agarose + herbicide solutions (Table A-2) in four 11x11x2.8 cm germination boxes (156C container, Hoffman Manufacturing

Inc; Corvallis, OR 97330 USA) and placed in growth chambers with 12 h of light with a temperature regime of 23/15 ºC for 10 days. Germination was counted, and seedling area was measured using Image analysis as previously described through the software ImageJ but for this assay, camera lens was set at 25 cm above from the seeds. Data were modelled with the following log-logistic regression equation [1] to obtain ED-50 values,

$$y = c + \frac{d-c}{1+\left(\frac{x}{ED_{50}}\right)^{b}} \quad [1]$$

Where *y* is the response, *d* refers to the upper limit and *c* to the lower limit of the sigmoid curve, $ED_{50}$ denotes the dose, x refers to the necessary rate to reduce 50% of the seedling area and germination between *d* and *c,* and *b* refers to the relative slope of the curve around $ED_{50}$ . The mean of these values for each herbicide treatment were used as discriminant rates for the seed screening assay.

A complete randomized block design with four replications were placed in 11x11x2.8 cm germination boxes (156C container, Hoffman Manufacturing Inc; Corvallis, OR 97330 USA) containing the gel medium plus herbicide. Germination was counted and image analysis were made 10 days after the start of the trial. Populations were classified in the three resistance presence levels as previously described.

## Ploidy level screening

DNA amount and ploidy level of each collected population were measured using an indirect approach by measuring the holoploid (2C) [28] relative genome with a flow cytometer[29–32]. Fifty seeds of each population were placed in 24 by 24 cm square plastic square trays and germinated in growth chambers set for 12 h of light with a temperature regime of 23/15 º C. Plants were watered daily and treated once a week with a standard

20-20-20 fertilizer (Miracle-Gro water soluble; The Scotts Company LLC, Maryville Ohio, USA). Eighteen days after germination, 6 plants (replications) from each population were randomly selected. Two expanded leaves were selected and approximately 1.5 $cm^2$ were sampled from each leaf. For each sample, leaves from a reference genome, tomato (*Solanum lycopersicum*), which has a 2C-value of 1.96 picograms (pg) were added to each sample. This species was chosen as a reference because it has a 2C-value smaller than a diploid Italian ryegrass, allowing the differentiation of peaks between tetraploid and diploid ryegrasses and the calculation of holoploid relative genome size (Figure A.4). Each leaf sample was chopped using a razor-blade in the presence of 400 ml of a buffer solution (Cystain Ultraviolet Precise P Nuclei Extraction Buffer; Sysmex, Gorlitz, Germany). The chopped leaf plus buffer solution was placed into a gauze filter with pore size of 30 mm and then placed in a 3.5 ml plastic tube. A fluorochrome stain (DAPI – 4', 6-diamidino-2-phenylindole) was added to the solution (Cystain Ultraviolet Precise P Staining Buffer; Partec). For analysis of the samples, a flow cytometer was used (CyFlow Ploidy Analyzer; Partec) calibrated to analyze at least 3,000 nuclei per sample. To calculate the 2C-value a DNA pg equation was used [2]. The results were compared with the 2C value mean values from two references cultivars of Italian ryegrass, the diploid 'Gulf' [33] and the tetraploid 'TAMTBO'.[34]

$$2C\ value\ (DNA\ pg) = Reference\ 2C\ value \times \frac{sample\ 2C\ mean\ peak\ position}{reference\ 2C\ mean\ peak\ position} \quad [2]$$

## Data analysis

Maps were constructed using ArcGIS and Oregon spatial data (Oregon spatial data library (https://spatialdata.oregonexplorer.info/geoportal/). Field points and shape files were projected to World Geodetic System 84 (WGS 84). Data were analyzed and organized using R software (https://www.R-project.org/) within the package collection Tidyverse (Hadley W (https://CRAN.R-project.org/package=tidyverse) and the package Survey [35] for inclusion of survey design and study area for frequencies calculation. A binomial logit regression was fitted to the model for the odds ratio of Italian ryegrass, overall resistance, multiple resistance (considering only high presence of resistance), and cross resistance (considering only high presence of resistance) presence with respect to elevation, plant density and crop type. The model was subjected to a Wald test to check the overall effects of each response variable. The packages aod (Lesnoff, M , Lancelot, R (https://cran.r-project.org/package=aod) and stats (R Core Team (https://www.R-project.org/) were used for fitting the models. A nearest neighbor analysis was conducted using the ArcGIS toolbox to test for significant cluster pattern of Italian ryegrass presence and resistance presence. The cluster patterns were classified by the algorithm as random, clustered or dispersed. The distance between fields was measured using a Euclidian distance and a study area of 50,000 hectares (grass and wheat areas combined in the surveyed area according to 2017 USDA/NASS spatial data [21]) using equation [3] to calculate the *z*-statistic value:

$$z = \left[ \frac{\bar{d} - \sum(d_i)}{\sqrt{0.0683x \frac{A}{N^2}}} \right] \qquad [3]$$

where *d* refers to the nearest neighbor distance, *A* refers to the study area and *N* to the number of points. Cluster analysis for multiple and cross resistance was made only considering populations with high presence of resistance to understand.

# Results

## Italian ryegrass presence

Of the 150 fields surveyed in 2017 and 2018, Italian ryegrass was found in 50% (75 fields) of the fields with a statistically significant larger percentage in fields surveyed in the year of 2017 (Figure 2.3). A similar distribution of Italian ryegrass presence was observed over the strata division (north, center and south) (Table 2.4). Italian Ryegrass presence per county showed similar patterns among the counties with Benton and Marion counties having larger numbers of surveyed fields (Table A.3). Forty percent of the tall fescue fields and 69% of the wheat fields had the Italian ryegrass present (Table 2.5). The results of the fitted binomial logit regression for Italian ryegrass presence (Table 2.11) had a *p*-value of 0.029 for the Wald test for the fitted model indicated that the overall effect of type of crop is significant for the presence of the weed and that the odds-ratio of Italian ryegrass is greater in wheat and orchardgrass than in tall fescues fields by the factor of 3.12 and 4.93, respectively.

## Italian ryegrass density

In the fields where Italian ryegrass was present, around 42% of the fields had a high plant density (more than 20 plants/$m^2$) with a larger percentage in 2017. Third-six percent of the surveyed fields had low density (9 or less plants/$m^2$) (Figure 2.4). With respect to stratum level, the frequency of high density was equally distributed among the stratum

and low density and medium density were lower on the south stratum (Table 2.6). In most of the crops, the predominant level was high density with exception of tall fescue fields that had 48% of the fields with low density level of Italian ryegrass (Table A.4). The distribution and frequency of density level per county showed a predominance of high-level density except at Linn and Yamhill counties.

## Ploidy level screening test

Flow cytometry results showed that there are populations of Italian ryegrass with tetraploid plants. In the 75 fields where ryegrass was present, 6.67% (5 populations) of the fields have tetraploid populations as weeds (Figure 2.5). These populations were found primarily in tall fescue fields (four populations) and one population was found in a wheat field. The average 2C in picograms from the diploid populations was 5.30 while the average size for tetraploid was 9.78 (Figure 2.29).

## Herbicide resistance presence

No resistance was found in the tetraploid populations. The results from the herbicide resistance screening showed that the overall frequency of populations with at least one type of an herbicide resistance (high or low resistance presence) was 88% (66 populations) of the tested populations (Figure 2.6). Populations of Italian ryegrass with multiple-resistance, defined as a population that exhibited resistance to more than one mode of action, represented 75% (56 populations) of the tested populations with no differences of frequency between the years (Figure 2.7). Cross-resistance, defined as resistance to more than one chemical with the same mode of action, frequency was 67% (50 populations) of the tested populations with similar percentages among the two years

(Figure 2.8). Results from binomial logit regression for overall herbicide resistance presence did not show any interaction with crop, density, location or elevation (Table 2.12).

## Types of resistance & modes of action

A frequency of 61% (46 populations) of the populations exhibited both cross and multiple resistance in the same population (Figure 2.28). Twenty-seven percent (21 populations) of the tested populations had a high level of plant density and exhibited both cross and multiple-resistance (Figure 2.31). Twenty-nine percent (22 populations) had both cross and multiple resistance and were located in the north region, 20% (15 populations) in the center and 8% (9 populations) in the south region (Figure 2.34).

The frequency of crop type data indicated that for most of the crops surveyed, the most common resistance type was cross and multiple resistance combined. Fifty percent (20 fields) of 40 tall fescue fields had multiple and cross resistance present while for the 18 surveyed wheat fields 88% (16 fields) had both multiple and cross resistance (Table 2.9).

Wald test and binomial logit results did not show significance effects on the odds ratio for cross and multiple resistance presence. Conversely, Wald test results for multiple resistance presence considering only the populations with high resistance presence showed overall correlation with plant density level. The binomial logit regression indicated that the odds-ratio of multiple resistance to be present in populations with high and medium density is greater than in populations with low density by the factor of 5.27 and 7.50, respectively (Table 2.13). The binomial logit regression results showed that the odds-ratio of finding cross-resistance (only considering high presence of resistance) in

wheat fields compared to tall fescue fields increased by a factor of 10.34. However, the Wald test did not elucidate any overall relationship between crop type and cross-resistance when considering only high presence of resistance (Table 2.14).

The most frequent modes of action (MOA) where resistance was documented were acetyl-CoA carboxylase (ACCase), acetolactate synthase (ALS) and 5-enolpyruvylshikimate-3-phosphate (EPSPs) inhibitors and combinations of them (Figure 2.20 to 26). Twenty three percent of the populations had resistance to both ALS and ACCase herbicides (Figure 2.30). The most common combinations of multiple resistance were ALS + ACCase, very long fatty-acid chain inhibitor (VLFA) + ALS + ACCase, EPSPs + ALS + ACCase and EPSPs + ALS (Table 2.3). Other combinations of modes of action were also found (Table A-5).

## Resistance level to tested herbicides

Of the 11 herbicides tested, only three herbicides had high presence of resistance in Italian ryegrass populations. (Figure 2.27). For ACCase inhibitor herbicides, all of them showed a greater number of populations with high resistance presence than the other levels of resistance and with a uniform distribution along the surveyed area (Figure 2.9 to 2.14).

For clethodim, 11% (8 populations) had high presence of resistance and 21%(16 populations) had low resistance presence. The frequency per crop data showed that no high clethodim resistance presence was found in the populations from tall fescue fields but there were 23% (9 fields) of tall fescue fields with low resistance presence (Table 2.7). Conversely, 28% (5 fields) of the wheat fields had populations with high presence of resistance (Table 2.8). Resistance was found in clover and orchardgrass fields (Table

A.6 to A.9). Eight percent (6 populations) of the tested populations came from fields with a high density of Italian ryegrass and have high presence of resistance to clethodim (Figure 2.32). The north stratum had a greater frequency of high presence of clethodim resistance (Figure 2.33).

Pinoxaden and quizalofop-p-ethyl results had greater frequencies of high presence of resistance level 37 and 40% (28 and 30 populations) of the tested populations, respectively. Both herbicides had a frequency around 20% (15 populations) of populations with low presence of resistance to this herbicide. The resistance presence to these herbicides with respect to the plant density results indicated similar distribution among the density levels (Figure 2.32). In the tall fescue fields where Italian ryegrass was present, 28 and 40% (11 and 16 tall fescue fields) had populations with high resistance presence to pinoxaden and quizalofop-p-ethyl, respectively (Table 2.7). In other crops orchardgrass and tree crop fields had a frequency of high presence of resistance of 20% for pinoxaden but no resistance to Quizalofop-p-ethyl (Table A.6 to A.9). The north region had greater frequencies for resistance to both herbicides (Figure 2.33).

Resistance to glyphosate is spread throughout the Willamette Valley; (Figure 2.12) however, not at the same frequency as for the ACCase herbicides. The overall frequency in the tested populations was 28% (21 populations) with high resistance presence and 15% (11 populations) with low resistance presence. High resistance presence was found in all three levels of plant density; 15% (11 populations) of the tested populations that have high resistance level to glyphosate had a high plant density level (Figure 2.32). A greater number of cases of resistance were found in the north stratum where 20% (15 populations) of the populations tested with high presence of resistance to glyphosate.

(Figure 2.33). The frequency of high presence of glyphosate resistance populations was 25% (10 fields) in the tall fescue fields with 13% (5 fields) with low resistance presence (Table 2.7). Twenty two percent (4 fields) of the wheat fields with Italian ryegrass present had high presence of glyphosate resistance populations with another 22% (4 fields) with low presence of resistance (Table 2.8). In tree crops, 60% (3 fields) of the fields had high presence of glyphosate resistance and 20% (1 fields) had a low resistance presence. Forty percent of the orchardgrass fields (4 fields) with Italian ryegrass present had a high presence of glyphosate resistance (Table A.6 to A.9).

No high resistance presence to glufosinate was found in either years of the survey. However, two populations in the north stratum showed low resistance presence (Figure 2.13). One population was located in a tree crop field and the other in an orchardgrass field.

High presence of paraquat resistance was observed in only limited populations (Figure 2.15). Some cases of populations with low presence of resistance also were observed. The cases of high presence of resistance were located in the north and center strata (Figure 2.33) with all populations having a high level of plant density (Figure 2.32). All populations that showed resistance patterns to paraquat came from tree crops (Table A.6).

The two tested ALS inhibitor herbicides, mesosulfuron and pyroxsulam, had similar results to the ACCase herbicides showing a greater frequency of high resistance presence. Mesosulfuron and pyroxsulam cases of high and low resistance presence were distributed over the surveyed area. Approximately 45% (34 populations) of the populations tested had high presence of resistance to mesosulfuron while 36% (27 populations) had high

presence levels to pyroxsulam (Figure 2.15 to 19). Most of the ALS resistance cases had resistance to both herbicides. From the populations with high plant density, 21% (16 populations) had high presence of resistance to mesosulfuron while 19% (15 populations) had high presence to pyroxsulam (Figure 2.32). The north region had a greater presence of resistance when compared to south and center strata. Mesosulfuron high resistance presence was present in 36% (27 populations) of the populations from north stratum while pyroxsulam resistance was present in 24% (18 populations) (Figure 2.32). Tall fescue fields had a frequency of 33% (13 fields) of high presence resistance to mesosulfuron and 45% (18 fields) with low presence of resistance; the frequency in tall fescue fields with high presence of resistance to pyroxsulam was 18% and 33% (7 and 13 fields) low presence of resistance to pyroxsulam (Table 2.7).

For the populations that showed low resistance presence, all the herbicides tested had a random or dispersed pattern of distribution along the surveyed area. In wheat fields, no susceptible populations to mesosulfuron were found; 83% (15 fields) had high resistance presence and 17% (3 fields) had low resistance presence (Table 2.8). The other surveyed crops had presented similar frequencies of resistance to mesosulfuron and pyroxsulam (Table A.6 to A.9).

For the pre-emergent herbicides, flufenacet + metribuzin, pronamide and pyroxasulfone, cases of high resistance presence were only found to flufenacet + metribuzin. Regarding cases of low resistance presence, all three herbicides had cases (Figure 2.17 to 22). Pyroxasulfone had only one population with patterns of low presence of resistance while 13% (10 populations) of the tested populations were classified as having low presence of resistance to pronamide. The populations that had high resistance

presence to flufenacet + metribuzin had a similar distribution among the levels of plant density (Figure 2.32). These flufenacet + metribuzin resistant populations were more frequent in the north and south strata; 6% (5 populations) of the populations tested from the center stratum also had high presence of resistance (Figure 2.33). Twenty percent (8 populations) of the tall fescue fields, had high presence of resistance and 18% (7 fields) with low presence of resistance to flufenacet + metribuzin; pronamide and pyroxasulfone controlled most populations tested (Table 2.7). In the wheat fields, 17% (3 fields) of the tested populations showed high presence of resistance to flufenacet + metribuzin and 33% (6 populations) showed low presence of resistance; the only case of low resistance presence to pyroxasulfone was found in a wheat field (Table 2.8). High presence of resistance to flufenacet + metribuzin was documented in a tree crop and in oat fields but no cases in clover fields (Table A.8 to A.9).

## Nearest neighbor cluster analysis

The nearest neighbor cluster initial analysis indicated that general resistance, cross-resistance and multiple resistance were distributed in a random pattern. Conversely, multiple resistance only considering populations with high presence of resistance showed a 90% significance clustered pattern in the surveyed area (Table 2.15 to 31). With respect to the most frequent modes of action to what resistance were documented, all had a random distribution pattern across the surveyed area. The analysis for the resistance levels (high and low presence of resistance) to each tested herbicide showed that for the populations with high presence of resistance to mesosulfuron had cluster pattern in the surveyed area while all the other herbicides had a random or dispersed pattern to all levels of resistance across the Willamette Valley.

## Discussion

The results from this two-year survey provide some insights for the understanding of herbicide resistance. Italian ryegrass was evenly distributed across the surveyed area. Fifty percent (75 fields) of the fields had Italian ryegrass present; these results could serve support that this weed is one of the most troublesome weeds in the Willamette Valley region. These results also point to a lack of effectiveness of control and to a possible large presence of seeds in the soil seed bank. Some previous studies already approached this topic by studying the longevity of Italian ryegrass and other species' seeds. [36,37] Thus, new studies should focus on ways to understand Italian ryegrass seeds in seed banks and to quantify the presence of seeds. [38,39] The results also pointed to a larger chance of finding Italian ryegrass infestations in wheat fields when compared to tall fescue fields indicating a possible strong selection pressure on the management of this weed with this specific crop.

High Italian ryegrass density in the fields also confirmed the high abundance of this weed. This high density probably is a result of many years of plants escaping herbicide applications and other management tools. The results of this survey indicate that the odds-ratio of multiple resistance to be present is higher on populations with high plant densities. These findings agree with previous research that had showed that weed densities are correlated with resistance presence creating an increase in weed abundance.[16]

The results confirm an already known situation by growers regarding the spread of herbicide resistance. Populations with both low and high presence of resistance were distributed along the Willamette Valley indicating that resistance to many types of

herbicides is already established. Similar frequencies of resistance to other surveyed areas around the world were documented, indicating that Oregon is following the same patterns regarding the overuse of herbicides. [12,14,15,17]

These findings are a result of the effects of multiple and repetitive applications with the same type of herbicides and management tools over the years, indicating the necessity of new management approaches to control the spread of resistance. Despite the high level of resistance to multiple herbicides, some of the herbicides tested are still providing good control.

Even with the efficient controlled provided by glufosinate, the success of control of this herbicide is highly dependent of plant growth stage, light intensity and other weather conditions which possibly indicates why some populations were classified as having a low presence of resistance [40–42]. However, Oregon already documented a case of glufosinate resistance in Italian ryegrass. No cases of high presence of resistance were found to pyroxasulfone. This herbicide provided excellent control even with the most resistant populations. Only one plant in a single population germinated in the presence of the herbicide; however, evolution and selection of biotypes can occur. [43–46] The numerous cases of resistance to flufenacet + metribuzin indicate a necessity in a better understanding of the mechanism of resistance that was still not well characterized in the case of flufenacet. Future studies should also look into the mechanisms of resistance of populations carrying resistance to flufenacet + metribuzin.

The large number of populations classified as having low presence of resistance to most of the tested herbicides should receive the greatest attention. These populations indeed have resistance present. Yet, due to the low number of individuals carrying the

resistance, they could still be manageable. Previous research shows that to control and suppress the spread of resistance, action needs to be taken before the exponential increase of individuals carrying the trait in the population. [47,48] Thus, this should be a combined effort among farmers, academics, government and industry. Only with a well-planned management approach and constant monitoring, could the spread and evolution of these populations be minimized. Future studies should continue this annual survey to increase data and also create a continuous study for year-comparisons of herbicide resistance evolution. Other species such as roughstalk bluegrass (*Poa trivialis*) should be included in a survey so management approaches could be done before a similar scenario to Italian ryegrass takes place for this species.

Cases of multiple and cross resistance were the most frequent types of resistance observed on this survey. A large number of those cases were located in the north stratum; where most of the wheat fields surveyed were located. This large frequency in wheat fields is probably due to the large dependency on ALS and ACCase inhibitors herbicides on this crop, which possibly created a high selective pressure on this species. Previous research in different areas showed similar results. [49,50,16,15] The survey results also pointed to a strong relationship between ALS and ACCase resistance presence since most populations tested carried resistance to both modes of action. Wheat growers should consider other management approaches to control Italian ryegrass such as a larger focus on pre-emergent herbicides and non-chemical approaches.

With the increase in the adoption of cover crops in the mid-west of the United States, this recent scenario of herbicide resistance in Oregon could become an issue in seed sales from Oregon. The ploidy diversity results could provide a way to reduce this issue. This

was the first survey in Oregon regarding Italian ryegrass that described the ploidy diversity within the species. Interestingly, the results of this survey showed that the tetraploid Italian ryegrass populations were not resistant.

Cross incompatibility between tetraploids and diploids was already shown in other species due to the most common progeny of this cross being a triploid sterile. [51–53]. These compatibility issues were documented in previous studies with perennial ryegrass (*Lolium perenne* L.) where no barrier for fertilization was found; however, a large production of triploid seeds with low vigor and germination rates were produced. [54] Thus, other studies suggest that the formation of 2n eggs from the crosses between diploid and tetraploid perennial ryegrass can occur when diploid plants are fertilized with tetraploid pollen but in a much smaller segregation of tetraploid and very rare cases of triploid descendants. However, the tetraploid offspring was likely formed by the fertilization of unreduced eggs of the diploid plant forming meiotic polyploids.[10] These studies only reported results about the fertilization of diploid plants by 2n pollen and did not test reciprocal crosses; future studies are needed on this topic.

An increased adoption of tetraploid cultivars of Italian ryegrass in Oregon could be a way to ensure that crosses between diploid Italian ryegrass weeds and cultivars will be reduced avoiding a possible genetic contamination of the cultivar and the spread of herbicide resistance. This adoption would increase the presence of tetraploid eggs in the pollen cloud. However, more studies testing if this cross between the weed and crop is occurring. Future studies will be needed to test this hypothesis. Increasing adoption of tetraploid cultivars could also help in the identification of seed contamination by the use

of flow cytometric analysis to test samples to identify possible diploid Italian ryegrass seeds. [54] [10]

This research had some limitations regarding the herbicide resistance tests. As mentioned previously, glufosinate is highly dependent on light conditions which could have generated some false positives regarding low resistance presence level. Similarly, the pronamide test resulted in some seedlings that, even though the above ground parts grew normally, some symptoms such non root formation, could diminish the development of the plants; the low resistance presence cases should be retested for confirmation.

# Conclusions

To our knowledge, this is the first survey on herbicide resistance and ploidy diversity in Italian ryegrass in western Oregon. Cases of high presence of resistance were equally distributed across the surveyed area indicating that this is a general problem and not to specific locations. The effects of multiple and repetitive applications with the same type of herbicides and modes of action are more than clear; these results indicate the necessity of new management approaches to control the spread and evolution of resistance.

The herbicides glufosinate and pyroxasulfone still provided control options, however, if used as the only management tool, can create a high selection pressure for the evolution of resistant biotypes. Moreover, other tools must be added to the farmer's management toolbox such as mechanical control and ways to minimize the presence of Italian ryegrass seeds in the seed bank by preventing plants from producing seeds.

This survey quantified for the first time the presence of tetraploid Italian ryegrass indicating that tetraploid cultivar of Italian ryegrass could be weedy; conversely, no resistance was found on those populations. This information can be used as base for new hypotheses regarding the increased use of tetraploid cultivars to avoid cross pollination between the crop and weed by increasing the presence of 2n eggs in the pollen cloud.

This study was the first step for a continuous set of studies to understand and suppress herbicide resistance in western Oregon. It will serve as a basis for future studies regarding understanding the spread and evolution of resistance and how to minimize it. Other surveys should be conducted in the following years to create a multiple year database to allow further analysis to check the evolution of herbicide resistance in the long term.

# References

1. Zahid HJ, Robinson E, Kelly RL. Agriculture, population growth, and statistical analysis of the radiocarbon record. *PNAS*. **113**:931–5 (2016).

2. Bararpour MT, Norsworthy JK, Burgos NR, Korres NE, Gbur EE. Identification and biological characteristics of ryegrass (*Lolium* spp.) accessions in Arkansas. *Weed Science*. **65**:350–60 (2017).

3. Busi R, Gaines TA, Walsh MJ, Powles SB. Understanding the potential for resistance evolution to the new herbicide pyroxasulfone: field selection at high doses versus recurrent selection at low doses. *Weed Research*. **52**:489–499 (2012).

4. Brunharo CA, Hanson BD. Multiple herbicide–resistant Italian ryegrass [*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot] in California perennial crops: characterization, mechanism of resistance, and chemical management. *Weed Science*. **66**:696–701 (2018).

5. Ellstrand NC. Current knowledge of gene flow in plants: implications for transgene flow. *Philosophical Transactions: Biological Sciences*. **358**:1163–70 (2003).

6. Zapiola M, Campbell C, Butler M, Mallory-Smith C. Escape and establishment of transgenic glyphosate-resistant creeping bentgrass (*Agrostis stolonifera*) in Oregon, USA: a 4-year study. *Journal of Applied Ecology*. **45**:486–94 (2008).

7. Ellstrand NC, Prentice HC, Hancock JF. Gene flow and introgression from domesticated plants into their wild relatives. *Annual review of Ecology and Systematics*. **30**:539–63 (1999).

8. Sarangi D, Tyre AJ, Patterson EL, Gaines TA, Irmak S, Knezevic SZ, et al. Pollen-mediated gene flow from glyphosate-resistant common waterhemp (*Amaranthus rudis* Sauer): consequences for the dispersal of resistance genes. *Scientific Reports*. **7**:44913 (2017).

9. Martins BA, Sánchez-Olguín E, Perez-Jones A, Hulting AG, Mallory-Smith C. Alleles contributing to ACCase-resistance in an Italian ryegrass (Lolium perenne ssp. multiflorum) population from Oregon. *Weed science*. **62**:468–473 (2014).

10. Lamote V, Baert J, Roldán-Ruiz I, De Loose M, Van Bockstaele E. Tracing of 2n egg occurrence in perennial ryegrass (*Lolium perenne* L.) using interploidy crosses. *Euphytica*. **123**:159–64 (2002).

11. Hanzlik K, Gerowitt B. Methods to conduct and analyze weed surveys in arable farming: a review. *Agron Sustain Dev*. **36**:11 (2016).

12. Jasieniuk M, Ahmad R, Sherwood AM, Firestone JL, Perez-Jones A, Lanini WT, et al. Glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in California: distribution,

response to glyphosate, and molecular evidence for an altered target enzyme. *Weed Science*. **56**:496–502 (2008).

13. Hanson BD, Shrestha A, Shaner DL. Distribution of glyphosate-resistant horseweed (*Conyza canadensis*) and relationship to cropping systems in the Central Valley of California. *Weed Science*. **57**:48–53 (2009).

14. Culpepper AS, Whitaker JR, MacRae AW, York AC. Distribution of glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) in Georgia and North Carolina during 2005 and 2006. *J Cotton Sci*. **12**:5 (2008).

15. Owen MJ, Powles SB. Distribution and frequency of herbicide-resistant wild oat (*Avena* spp.) across the Western Australian grain belt. *Crop Pasture Sci*. **60**:25–31 (2009).

16. Hicks HL, Comont D, Coutts SR, Crook L, Hull R, Norris K, et al. The factors driving evolved herbicide resistance at a national scale. *Nature Ecology and Evolution*. **2**:529 (2018).

17. Keshtkar E, Mathiassen SK, Moss SR, Kudsk P. Resistance profile of herbicide-resistant *Alopecurus myosuroides* (black-grass) populations in Denmark. *Crop Protection*. **69**:83–9 (2015).

18. Avila-Garcia WV, Mallory-Smith C. Glyphosate-resistant Italian ryegrass (*Lolium perenne*) populations also exhibit resistance to glufosinate. *Weed science*. **59**:305–309 (2011).

19. Perez-Jones A, Park KW, Colquhoun J, Mallory-Smith C, Shaner D. Identification of glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in Oregon. *Weed Science*. **53**:775–779 (2005).

20. Betts KJ, Ehlke NJ, Wyse DL, Gronwald JW, Somers DA. Mechanism of inheritance of diclofop resistance in Italian ryegrass (*Lolium multiflorum*). *Weed Science*. **40**:184–189 (1992).

21. Boryan C, Yang Z, Mueller R, Craig M. Monitoring US agriculture: the US Department of Agriculture, National Agricultural Statistics Service, Cropland Data layer program. *Geocarto International*. **26**:341–58 (2011).

22. Ali A, Streibig JC, Duus J, Andreasen C. Use of image analysis to assess color response on plants caused by herbicide application. *Weed Technology*. **27**:604–11 (2013).

23. Ridler T, Calvard S. Picture thresholding using an iterative selection method. *IEEE trans syst Man Cybern*. **8**:630–2 (1978).

24. Tomasi C, Manduchi R. Bilateral filtering for gray and color images. In: Proceedings of theIEEE International Conference on Computer Vision; 1998 Jan 4; Bombay, India; (1998).

25. Beckie HJ, Heap IM, Smeda RJ, Hall LM. Screening for herbicide resistance in weeds. *Weed Technology*. **14**:428–45 (2000).

26. Murray BG, Friesen LF, Beaulieu KJ, Morrison IN. A seed bioassay to Identify Acetyl-CoA carboxylase inhibitor resistant wild oat (*Avena fatua*) populations. *Weed Technology*. **10**:85–9 (1996).

27. Tal A, Kotoula-Syka E, Rubin B. Seed-bioassay to detect grass weeds resistant to acetyl coenzyme A carboxylase inhibiting herbicides. *Crop Protection*. **19**:467–72 (2000).

28. Greilhuber J, Doležel J, Lysák MA, Bennett MD. The origin, evolution and proposed stabilization of the terms 'genome size' and 'C-value' to describe nuclear DNA contents. *Annals of Botany*. **95**:255–260 (2005).

29. Contreras RN, Shearer K. Genome size, ploidy, and base composition of wild and cultivated acer. *Journal of the American Society for Horticultural Science*. **143**:470–85 (2018).

30. Doležel J, Greilhuber J, Suda J. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols*. **2**:2233–44 (2007).

31. Lattier JD, Chen H, Contreras RN. Variation in genome size, ploidy, stomata, and rDNA signals in *Althea*. *Journal of the American Society for Horticultural Science*. **144**:130–140 (2019).

32. Rothleutner JJ, Friddle MW, Contreras RN. Ploidy levels, relative genome sizes, and base pair composition in *Cotoneaster*. *Journal of the American Society for Horticultural Science*. **141**:457–66 (2016).

33. Weihing RM. Registration of Gulf annual ryegrass (Reg. No. 8). *Crop Science*. **3**:366–366 (1963).

34. Nelson LR, Crowder J, Turner FT, Evers GW, Rouquette FM. Registration of 'TAMTBO' annual ryegrass. *Journal of Plant Registrations*. **1**:127–8 (2007).

35. Lumley T. Analysis of complex survey samples. *Journal of Statistical Software*. **9**:1–19 (2004).

36. Rampton HH, Ching TM. longevity and dormancy in seeds of several cool-season grasses and legumes buried in soil. *Agronomy Journal.* **58**:220–2 (1966 4/01).

37. Brewster DB, Donaldson SW, Mallory-Smith C. Longevity of dicoflop-resistant Italian ryegrass seed in soil. In: Vol. 48, Proceedings - Western Society of Weed Science Annual meeting; 1995 March 13-15; Sacramento, California; (1995).

38. Buhler DD, Hartzler RG, Forcella F. Implications of weed seedbank dynamics to weed management. *Weed Science*. **45**:329–36 (1997).

39. Davis AS. When does it make sense to target the weed seed bank? *Weed Science*. **54**:558–65 (2006).

40. Kumaratilake AR, Preston C. Low temperature reduces glufosinate activity and translocation in wild radish (*Raphanus raphanistrum*). *Weed Science*. **53**:10–16 (2005).

41. Ganie ZA, Jugulam M, Jhala AJ. Temperature influences efficacy, absorption, and translocation of 2,4-D or glyphosate in glyphosate-resistant and glyphosate-susceptible common ragweed (*Ambrosia artemisiifolia*) and giant ragweed (*Ambrosia trifida*). *Weed Science; Lawrence*. **65**:588–602 (2017).

42. Xie HS, Hsiao AI, Quick WA. Influence of temperature and light intensity on absorption, translocation, and phytotoxicity of fenoxaprop-ethyl and imazamethabenz-methyl in *Avena fatua*. *Journal of Plant Growth Regulation*. **15**:57–62 (1996).

43. Bagavathiannan MV, Davis AS. An ecological perspective on managing weeds during the great selection for herbicide resistance. *Pest Manag Sci*. **74**:2277–2286 (2018).

44. Bock DG, Kantar MB, Caseys C, Matthey-Doret R, Rieseberg LH. Evolution of invasiveness by genetic accommodation. *Nature Ecology & Evolution*. **2**:991 (2018).

45. Délye C, Jasieniuk M, Le Corre V. Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics*. **29**:649–58 (2013).

46. Powles SB, Yu Q. Evolution in action: plants resistant to herbicides. *Annual Review of Plant Biology*. **61**:317–47 (2010).

47. Evans JA, Tranel PJ, Hager AG, Schutte B, Wu C, Chatham LA, et al. Managing the evolution of herbicide resistance. *Pest Manag Sci*. **72**:74–80 (2016).

48. Hurley TM, Frisvold G. Economic barriers to herbicide-resistance management. *Weed Science*. **64**:585–594 (2016).

49. Owen MJ, Martinez NJ, Powles SB. Multiple herbicide-resistant *Lolium rigidum* (annual ryegrass) now dominates across the Western Australian grain belt. *Weed Research*. **54**:314–324 (2014).

50. Llewellyn RS, Powles SB. High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheat belt of Western Australia. *Weed Technology*. **15**:242–248 (2001).

51. Nichiyama I, Inomata N. Embryological studies on cross-incompatibility between 2x and 4x in *Brassica*. *The Japanese Journal of Genetics*. **41**:27–42 (1966).

52. Watkins AE. Hybrid sterility and incompatibility. *Journal of Genetics*. **25**:125–162 (1932).

53. Gill MS, Bajaj YPS. Hybridization between diploid (*Gossypium arboreum*) and tetraploid (*Gossypium hirsutum*) cotton through ovule culture. *Euphytica*. **36**:625–630 (1987).

54. Griffiths DJ, Pegler RAD, Tonguthaisri T. Cross compatibility between diploid and tetraploid perennial ryegrass (*Lolium perenne* L.). *Euphytica*. **20**:102–12 (1971).

# Figures

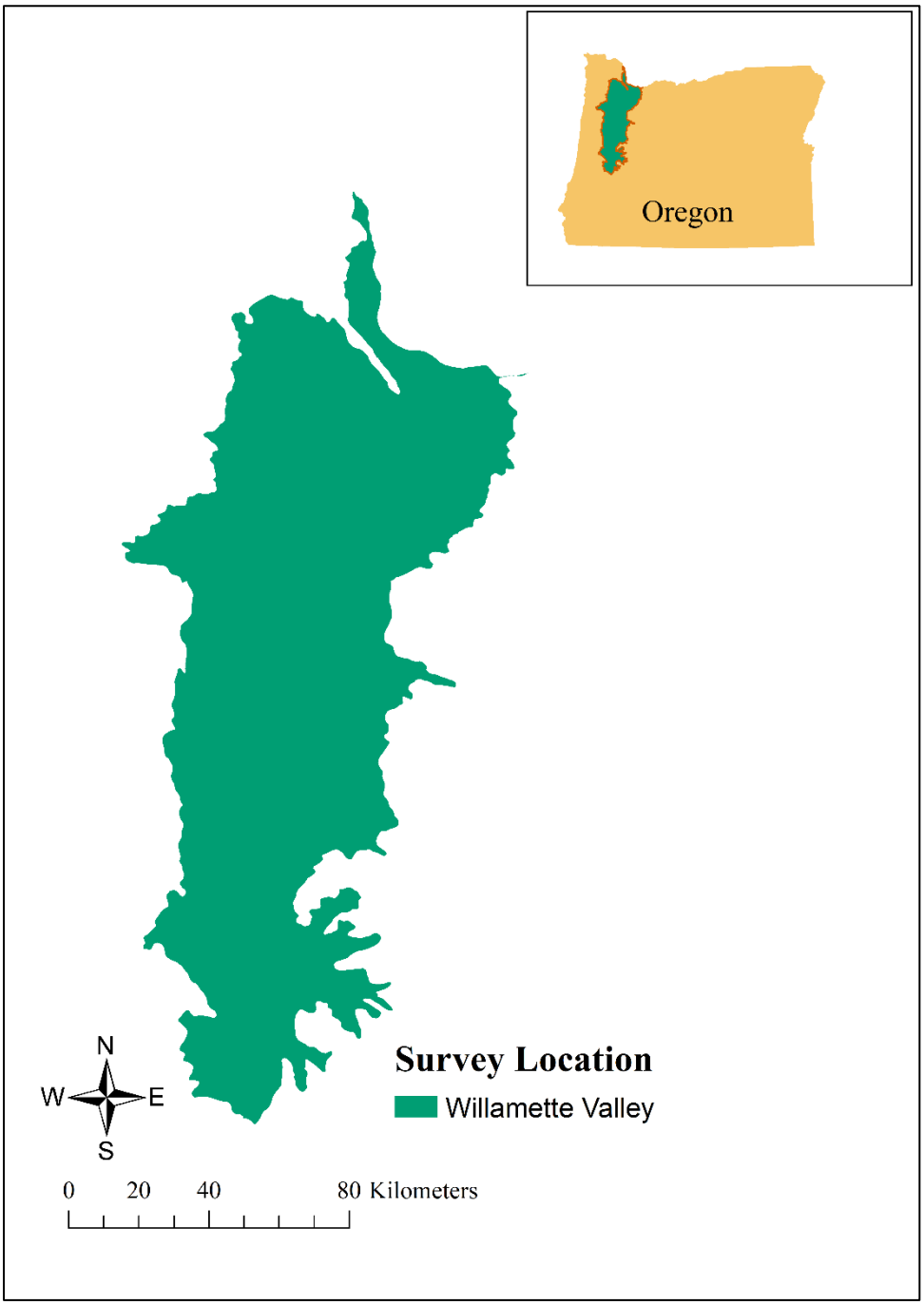



Figure 2-1. Survey location: ecoregion of the Willamette Valley in western Oregon

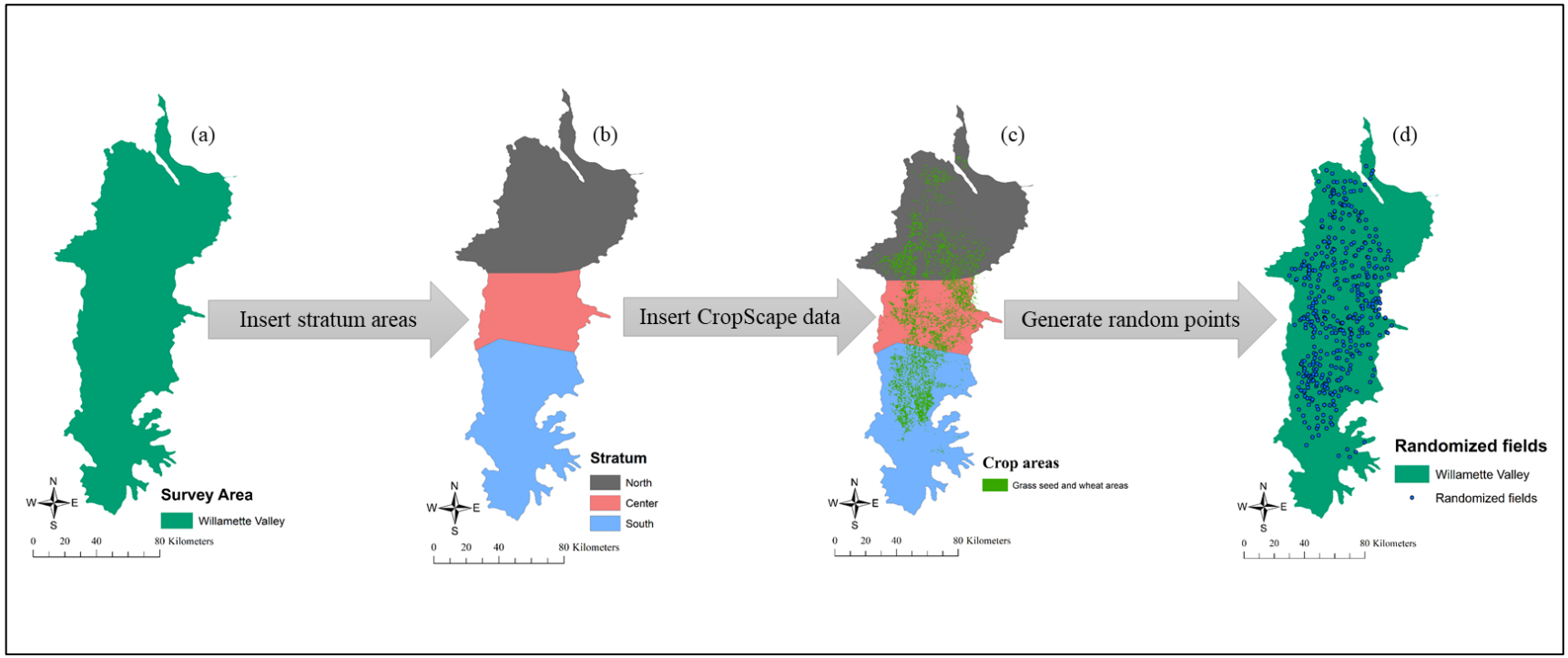



Figure 2-2 Survey Design - Stratified sampling design; (a) Willamette Valley area in green; (b) Stratification in three areas according to acreage (north in brown, center in pink and south in blue); (c) plotting the acreage data in green to be used as strata to randomly select points; (d) Randomly chosen points in blue on each stratum.

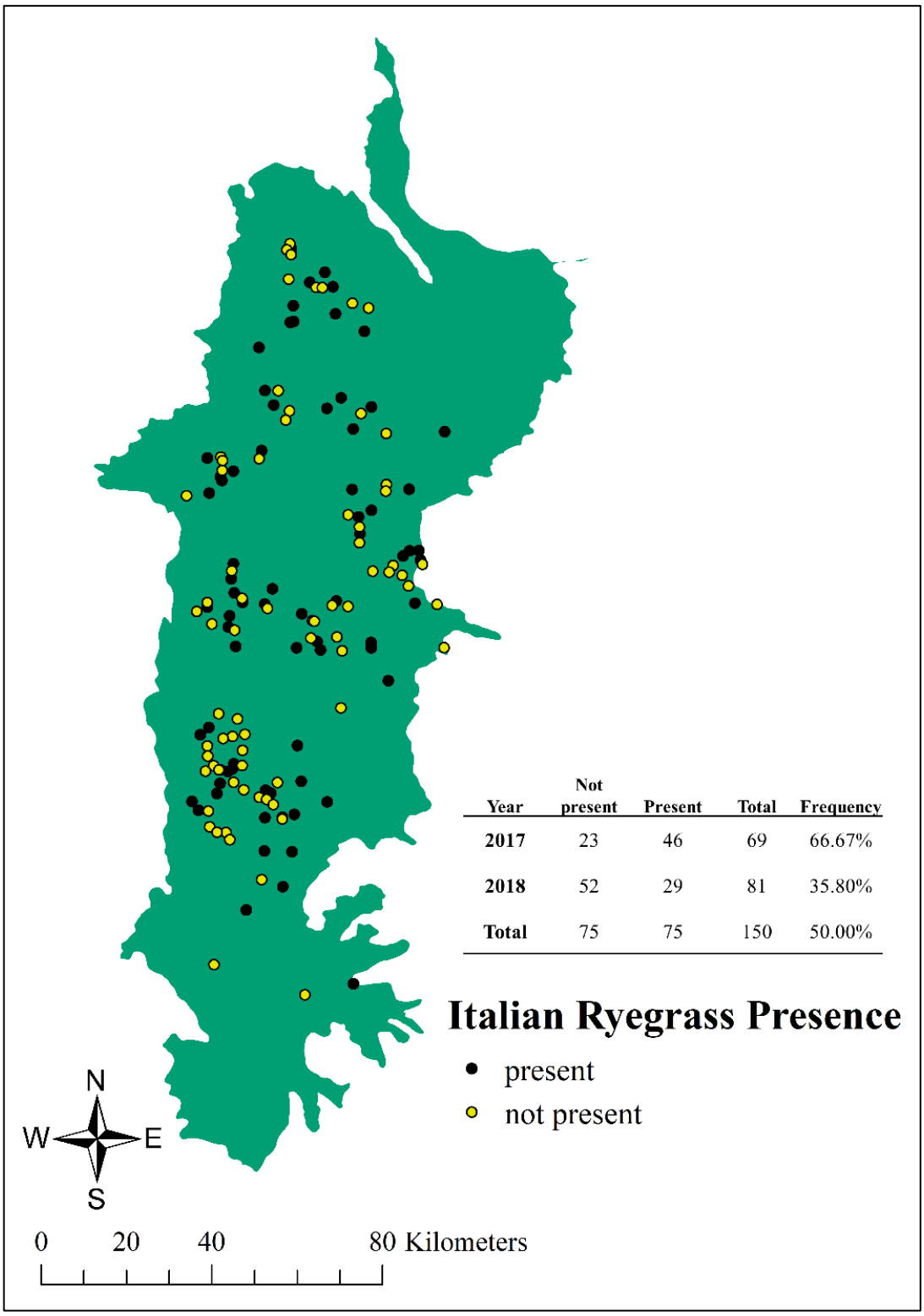
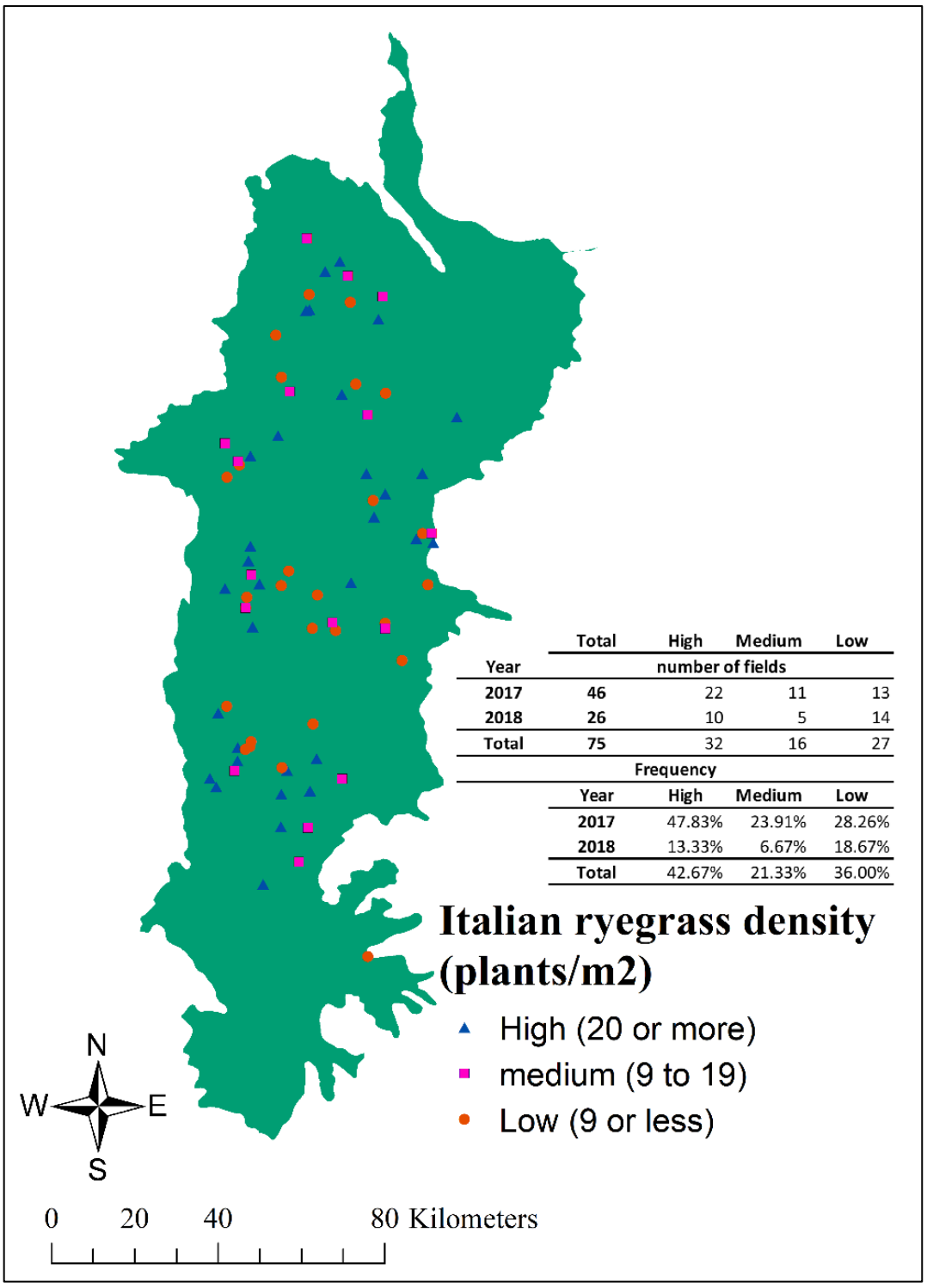



Figure 2-3. Italian ryegrass presence in both surveyed years and overall frequency.

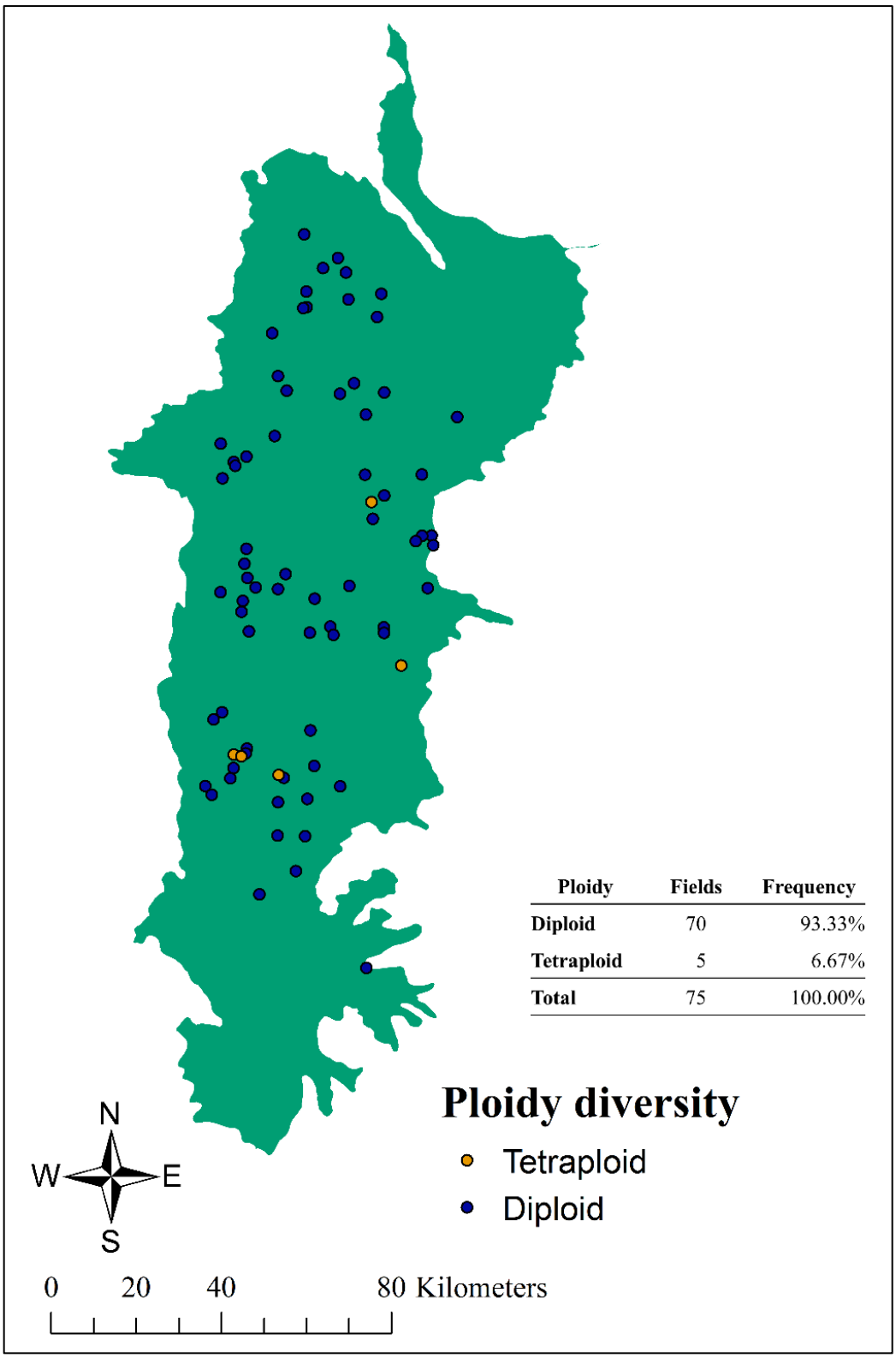



| Year | Total | High | Medium | Low |
|---|---|---|---|---|
| | number of fields | | | |
| 2017 | 46 | 22 | 11 | 13 |
| 2018 | 26 | 10 | 5 | 14 |
| Total | 75 | 32 | 16 | 27 |

Frequency

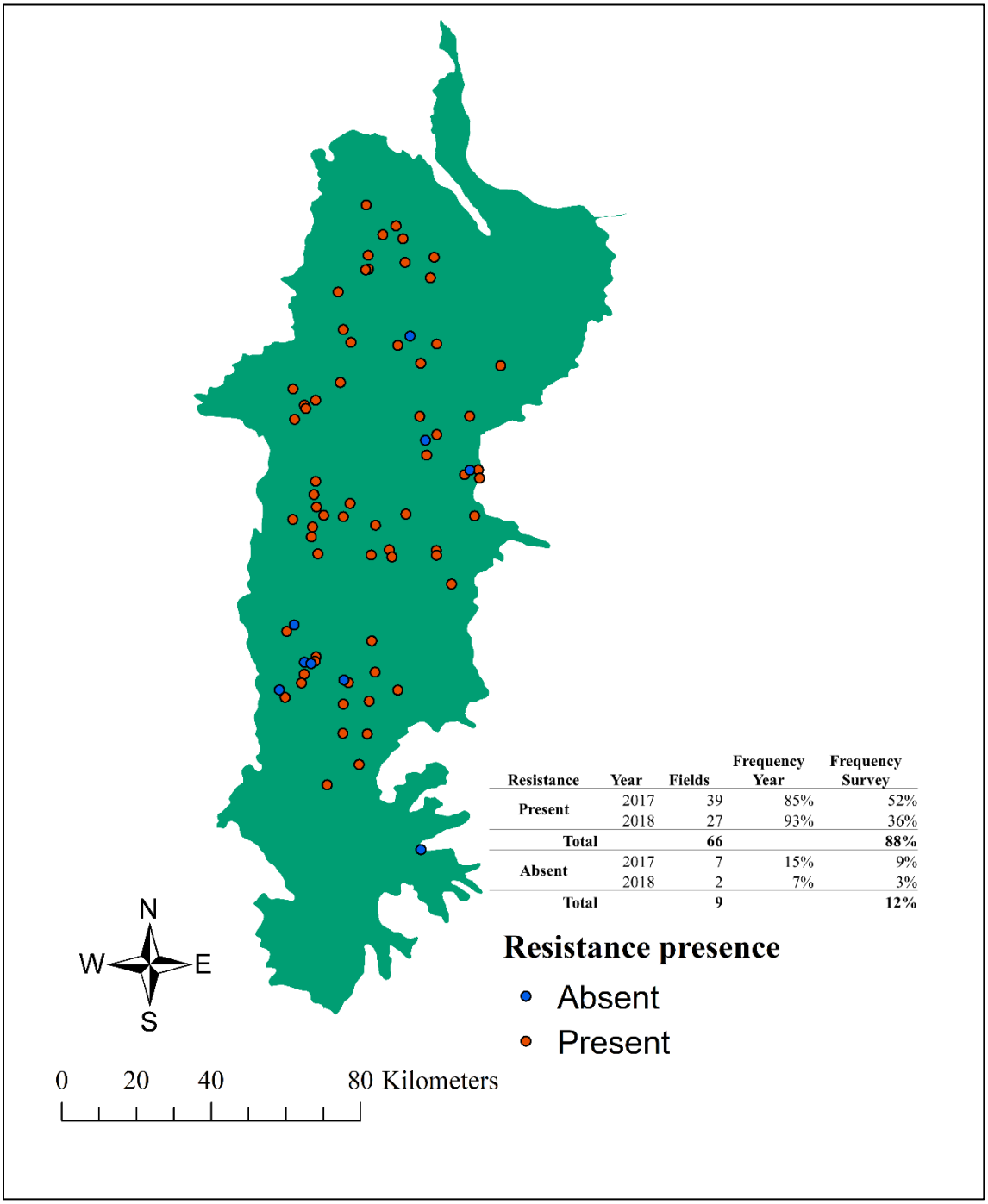
| Year | High | Medium | Low |
|---|---|---|---|
| 2017 | 47.83% | 23.91% | 28.26% |
| 2018 | 13.33% | 6.67% | 18.67% |
| Total | 42.67% | 21.33% | 36.00% |

Figure 2-4. Density levels of plants on locations where Italian ryegrass was present on the surveyed area. Ryegrass density was evaluated in three levels: high (20 plants or more per square meter), medium (10 to 19 plants per square meter) and low (1 to 9 plants per square meter).



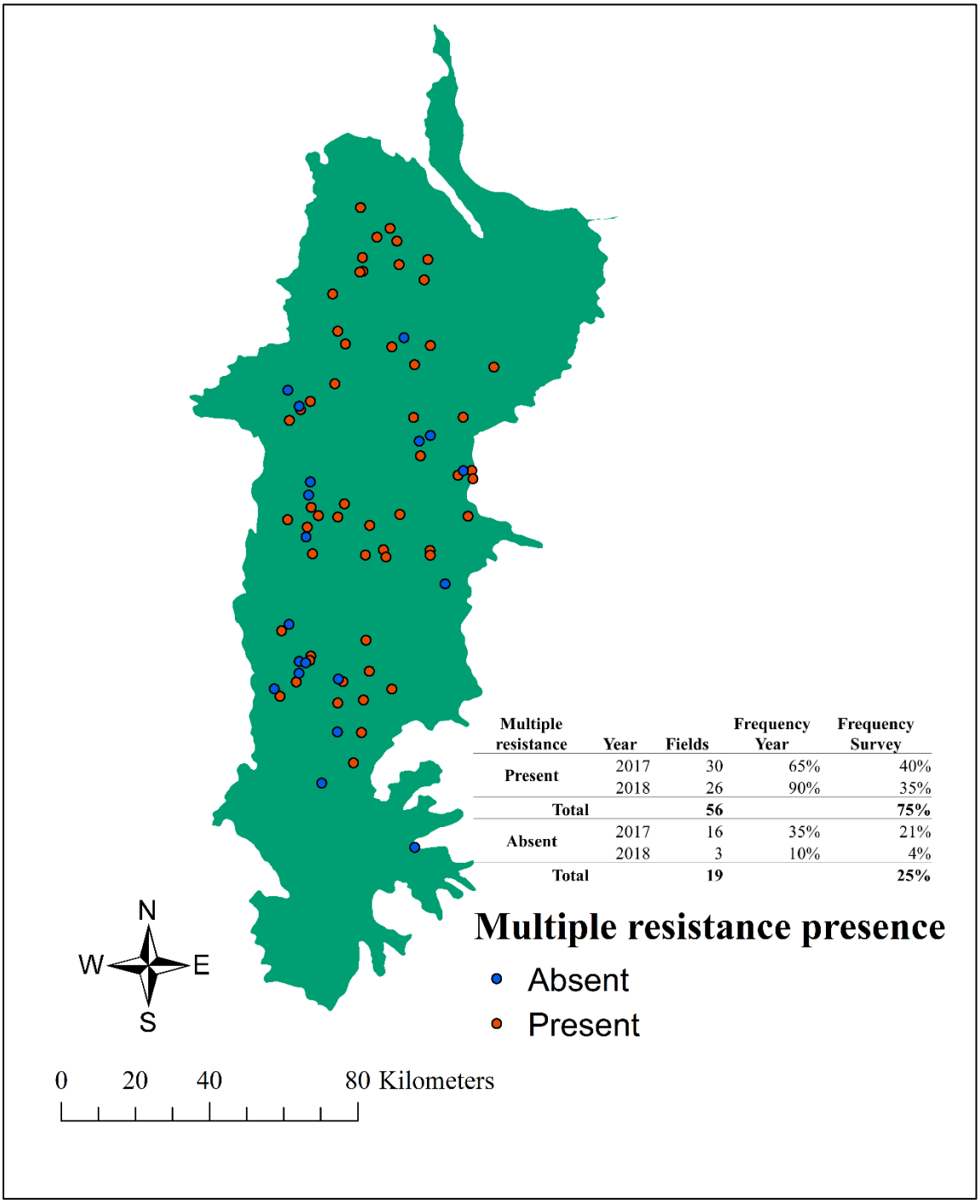

| Ploidy | Fields | Frequency |
|---|---|---|
| **Diploid** | 70 | 93.33% |
| **Tetraploid** | 5 | 6.67% |
| **Total** | 75 | 100.00% |

Figure 2-5. Ploidy diversity of Italian ryegrass populations distribution in the Willamette Valley.



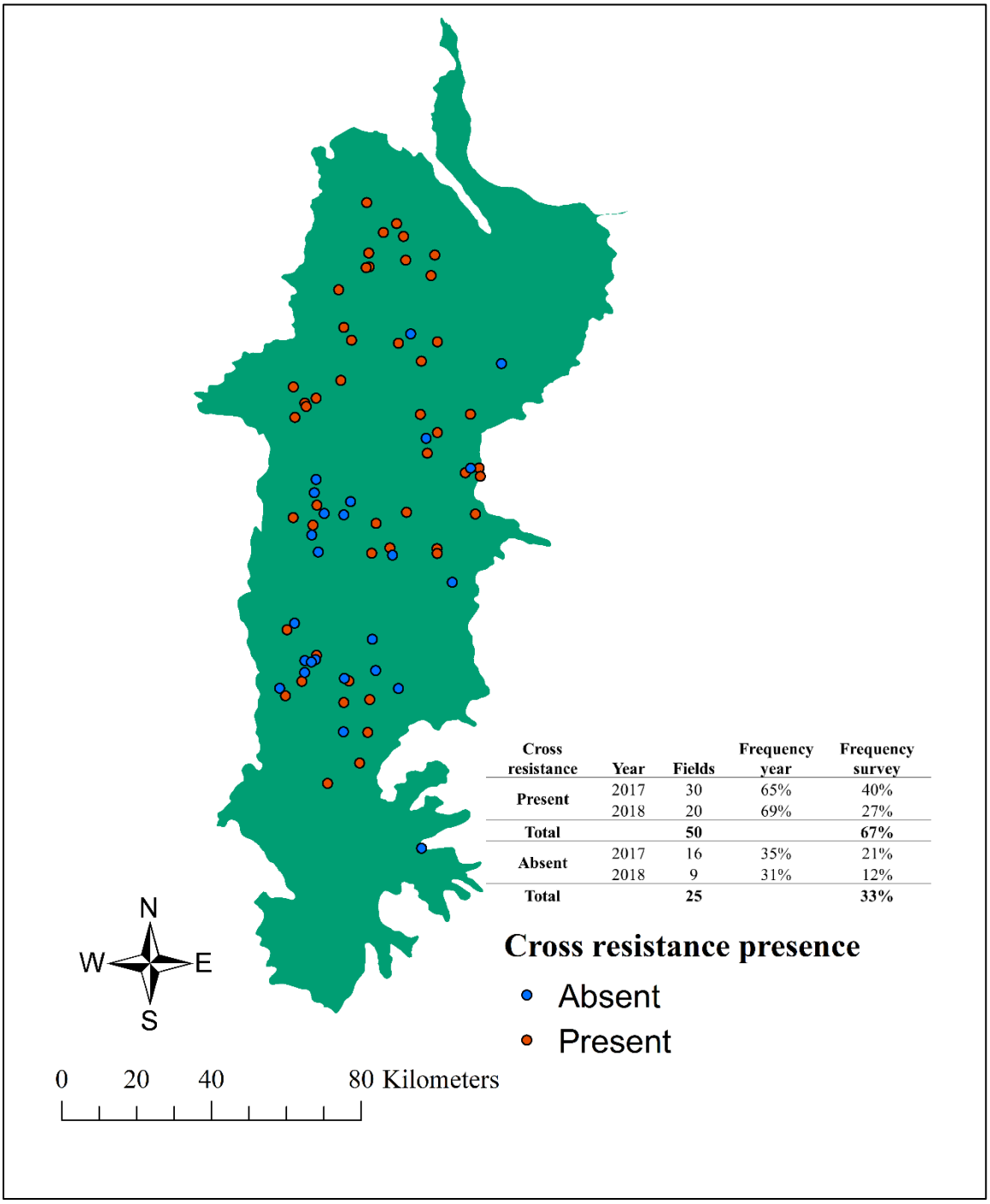

| Resistance | Year | Fields | Frequency Year | Frequency Survey |
|---|---|---|---|---|
| Present | 2017 | 39 | 85% | 52% |
| | 2018 | 27 | 93% | 36% |
| **Total** | | **66** | | **88%** |
| Absent | 2017 | 7 | 15% | 9% |
| | 2018 | 2 | 7% | 3% |
| **Total** | | **9** | | **12%** |

Figure 2-6. Population distribution and frequency where at least one case of high or low resistance presence documented per year and the overall survey.




| Multiple resistance | Year | Fields | Frequency Year | Frequency Survey |
|---|---|---|---|---|
| Present | 2017 | 30 | 65% | 40% |
| | 2018 | 26 | 90% | 35% |
| **Total** | | **56** | | **75%** |
| Absent | 2017 | 16 | 35% | 21% |
| | 2018 | 3 | 10% | 4% |
| **Total** | | **19** | | **25%** |

Figure 2-7. Multiple resistance presence distribution and frequency of each year and the overall in the Willamette Valley, OR.




| Cross resistance | Year | Fields | Frequency year | Frequency survey |
|---|---|---|---|---|
| Present | 2017 | 30 | 65% | 40% |
| | 2018 | 20 | 69% | 27% |
| Total | | 50 | | 67% |
| Absent | 2017 | 16 | 35% | 21% |
| | 2018 | 9 | 31% | 12% |
| Total | | 25 | | 33% |

Figure 2-8. Cross resistance presence distribution and frequency of each year and the overall survey in the Willamette valley, OR.

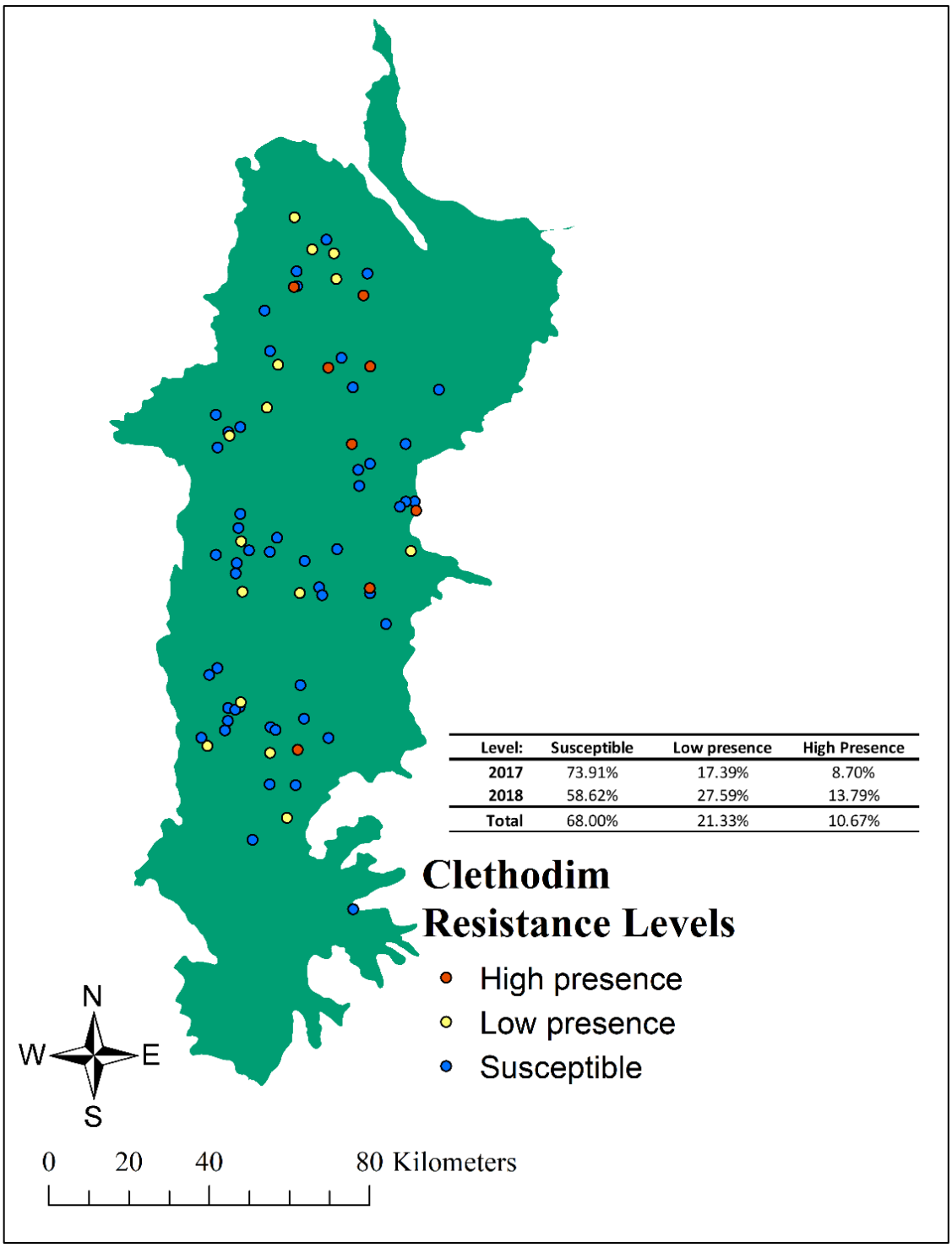



Figure 2-9. Distribution and frequency of resistance to clethodim in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

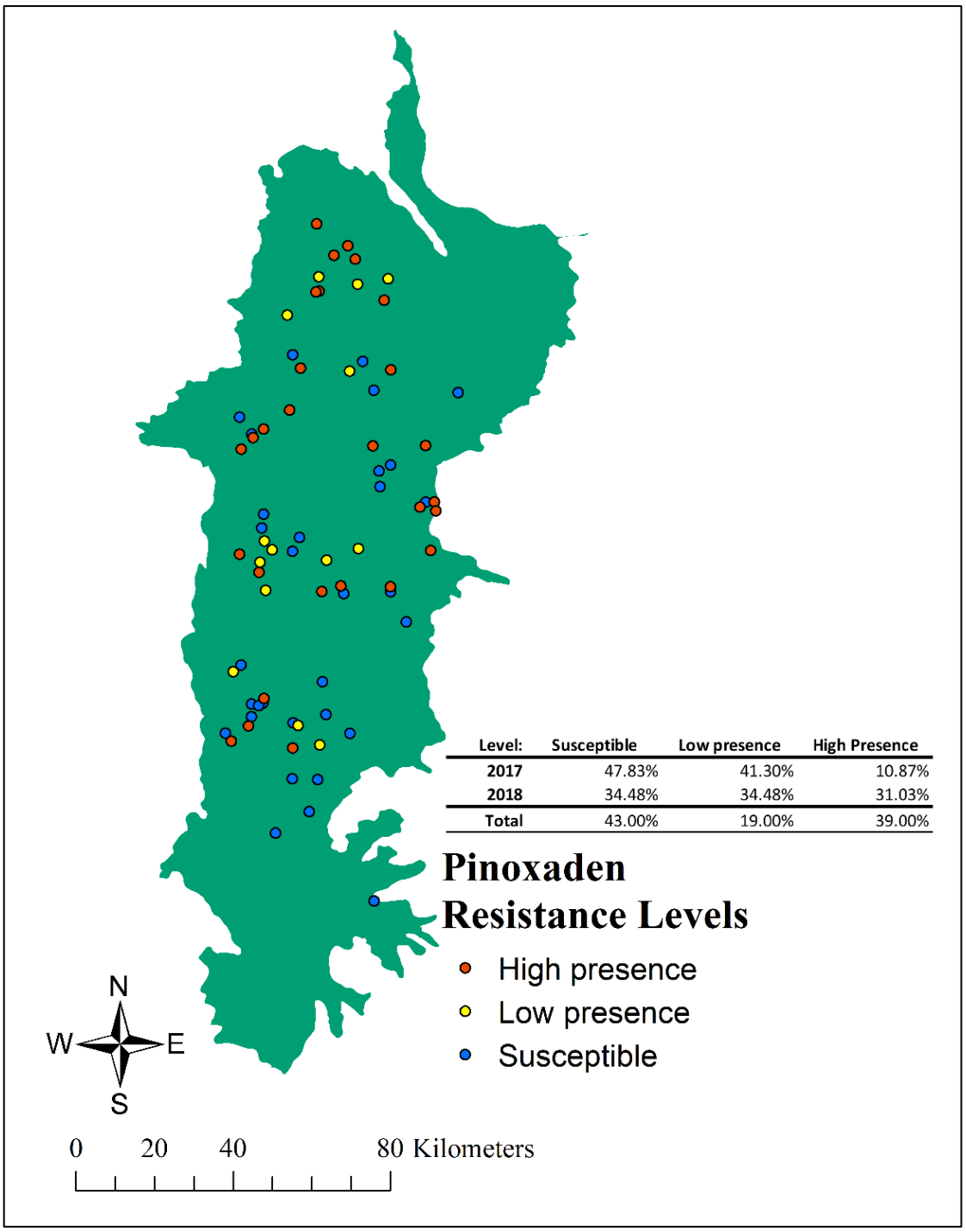



Figure 2-10. Distribution and frequency of resistance to pinoxaden in the Willamette Valley, OR. High presence in orange (20% or more survival and less than 70% green area reduction), low presence in yellow (2-19% survival and green area reduction between 70 to 90%) and susceptible in blue (less than 2% survival and green area reduction at least 90%).

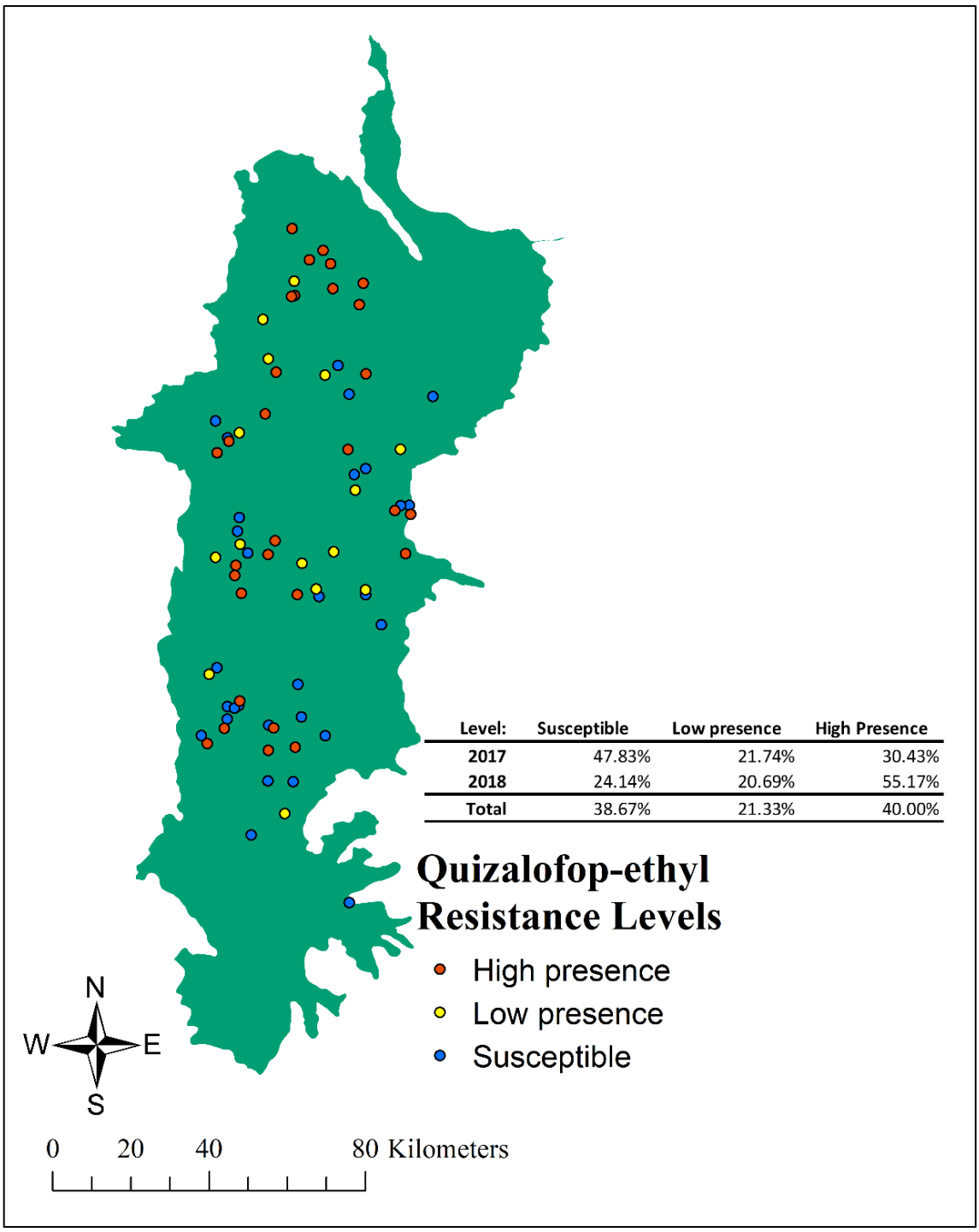



Figure 2-11. Distribution and frequency of resistance to quizalofop-p-ethyl in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

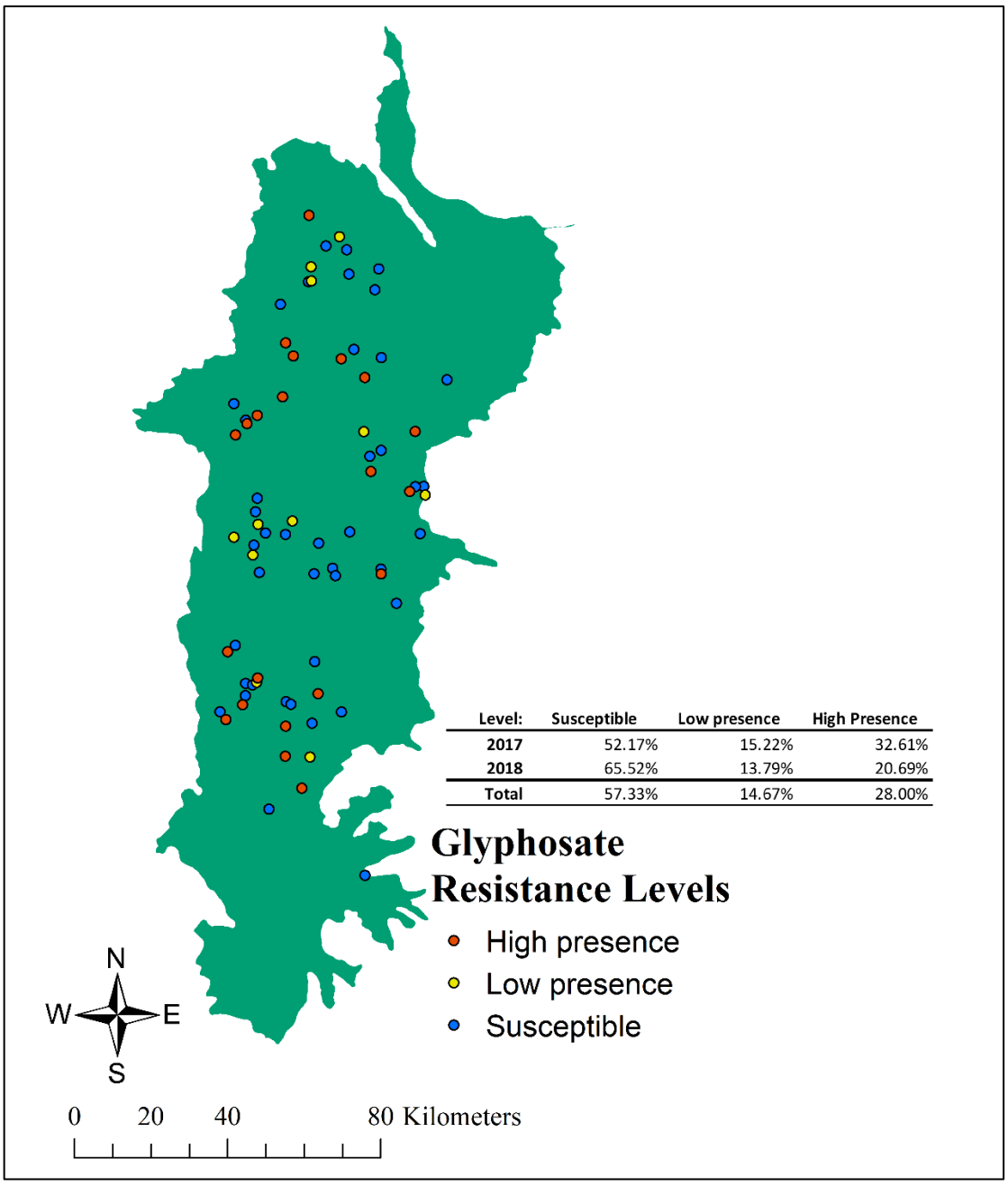



Figure 2-12. Distribution and frequency of resistance to glyphosate in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

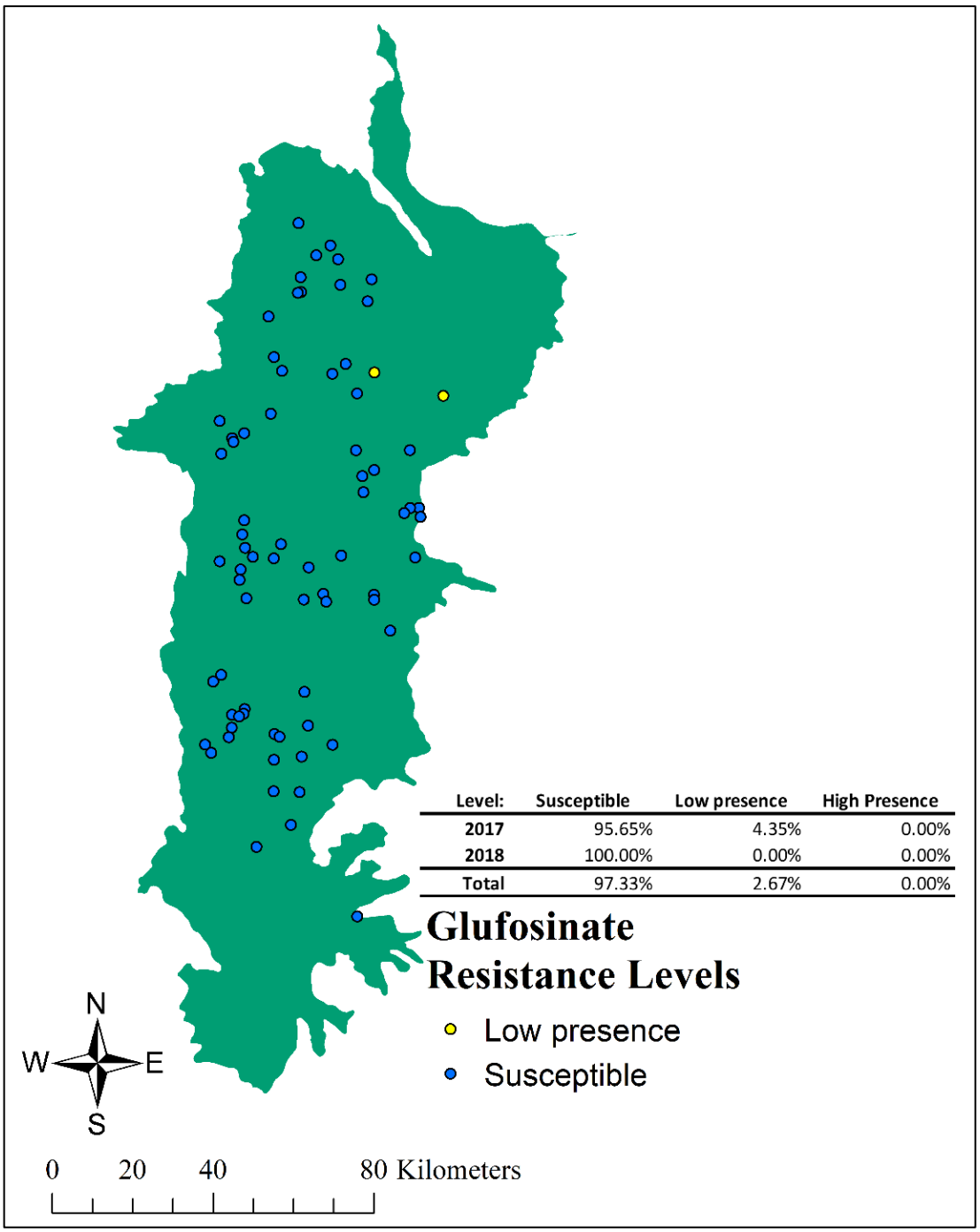



Figure 2-13. Distribution and frequency of resistance to glufosinate in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

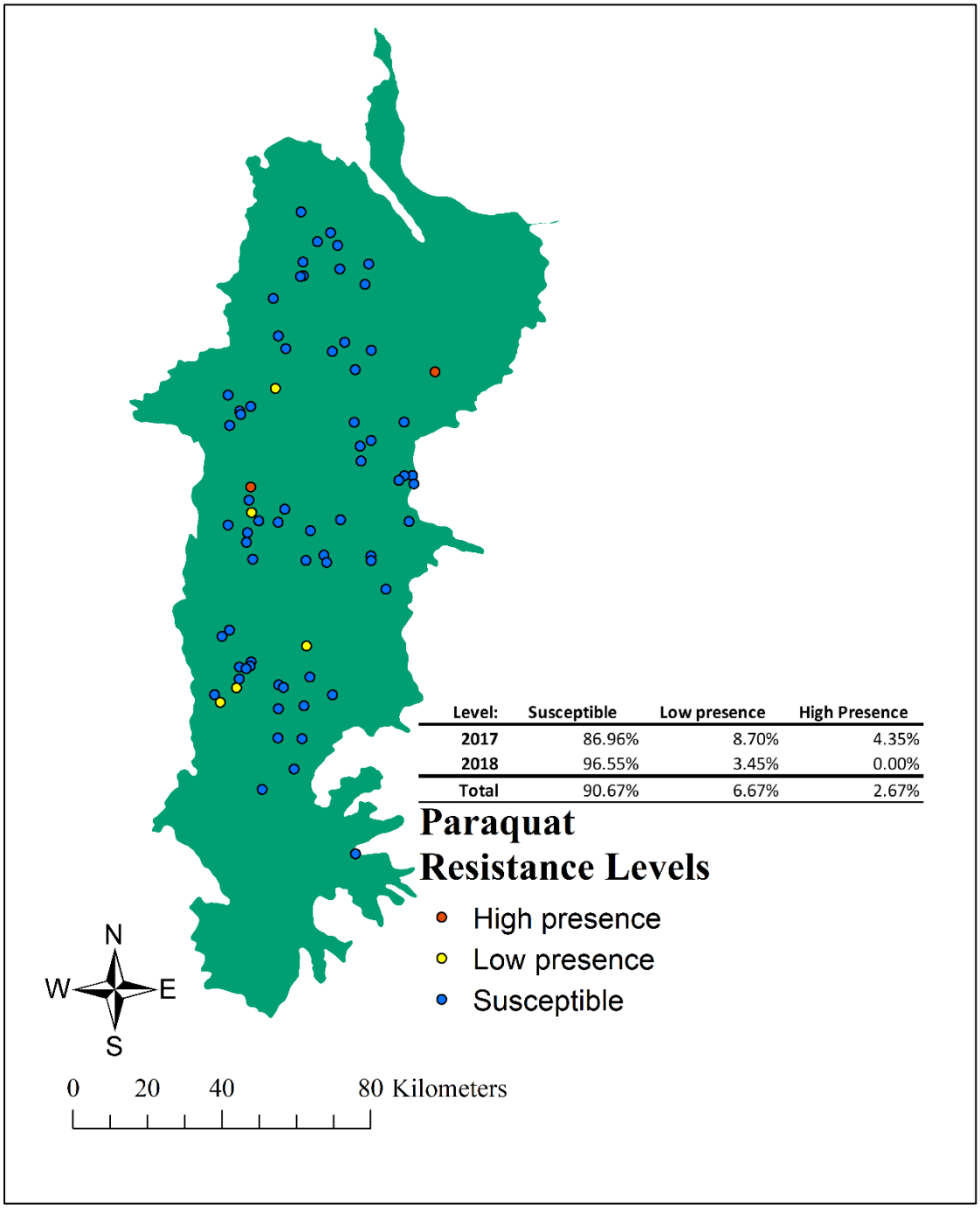



Figure 2-14. Distribution and frequency of resistance to paraquat in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

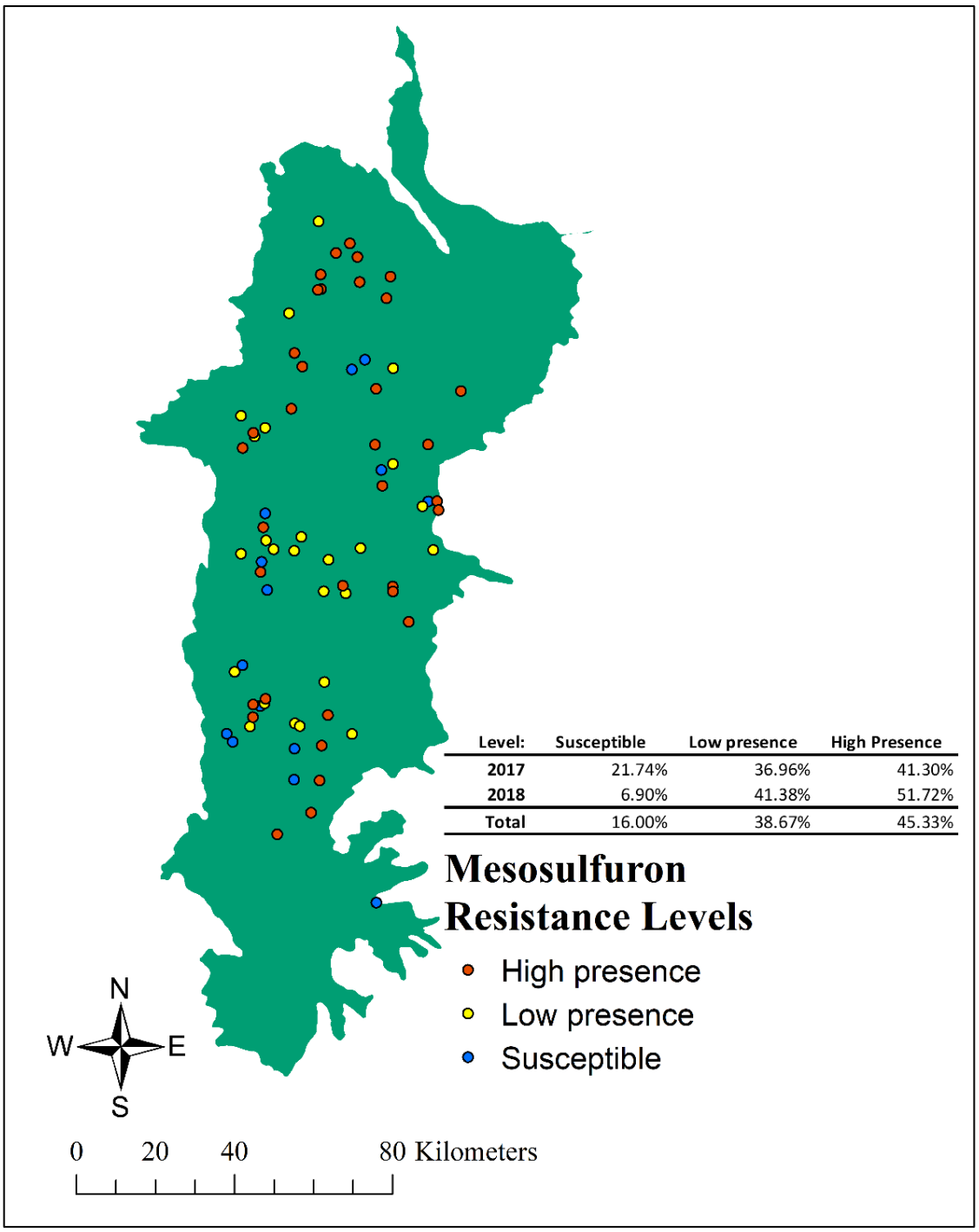



Figure 2-15. Distribution and frequency of resistance to mesosulfuron in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

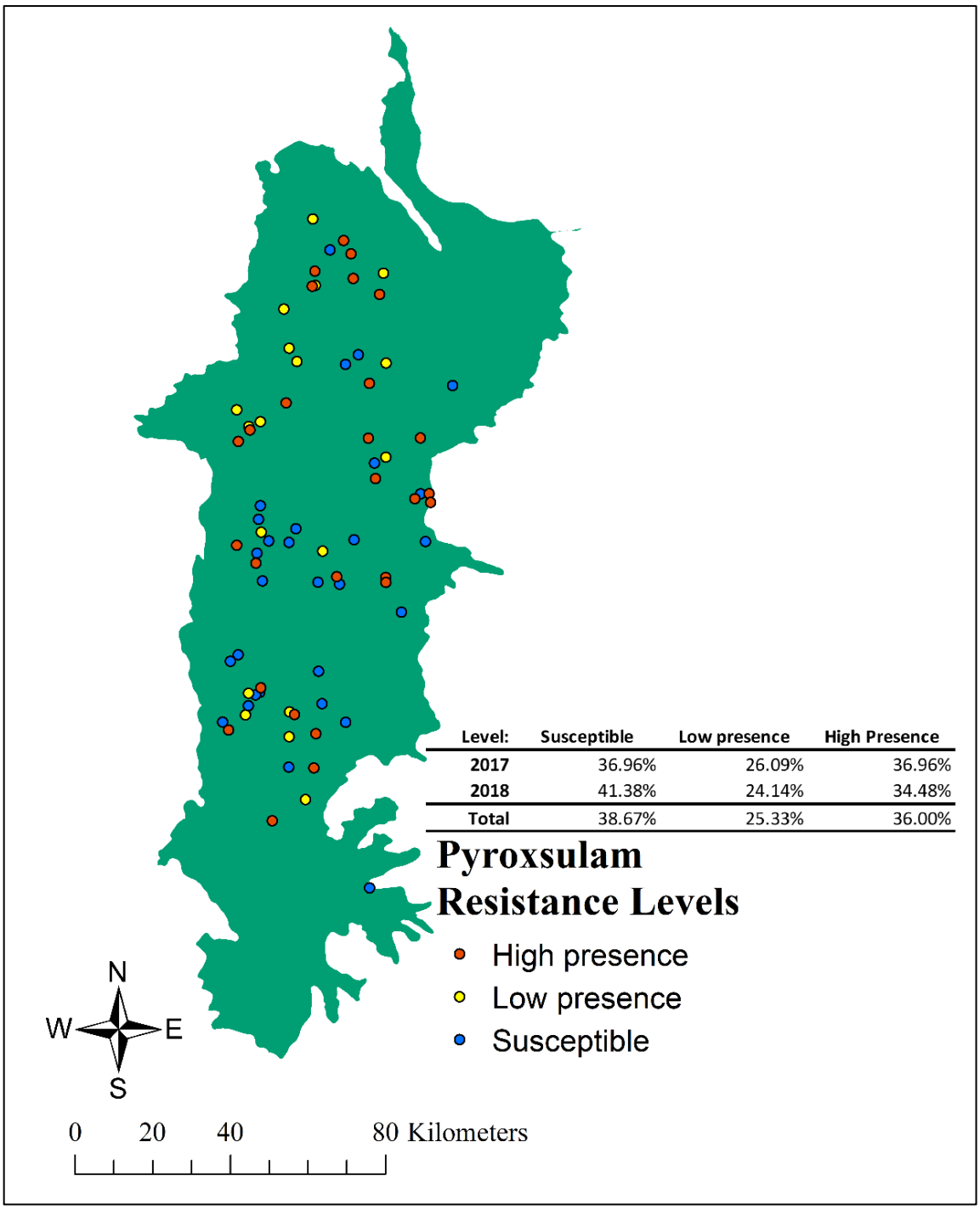



Figure 2-16. Distribution and frequency of resistance to pyroxsulam in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

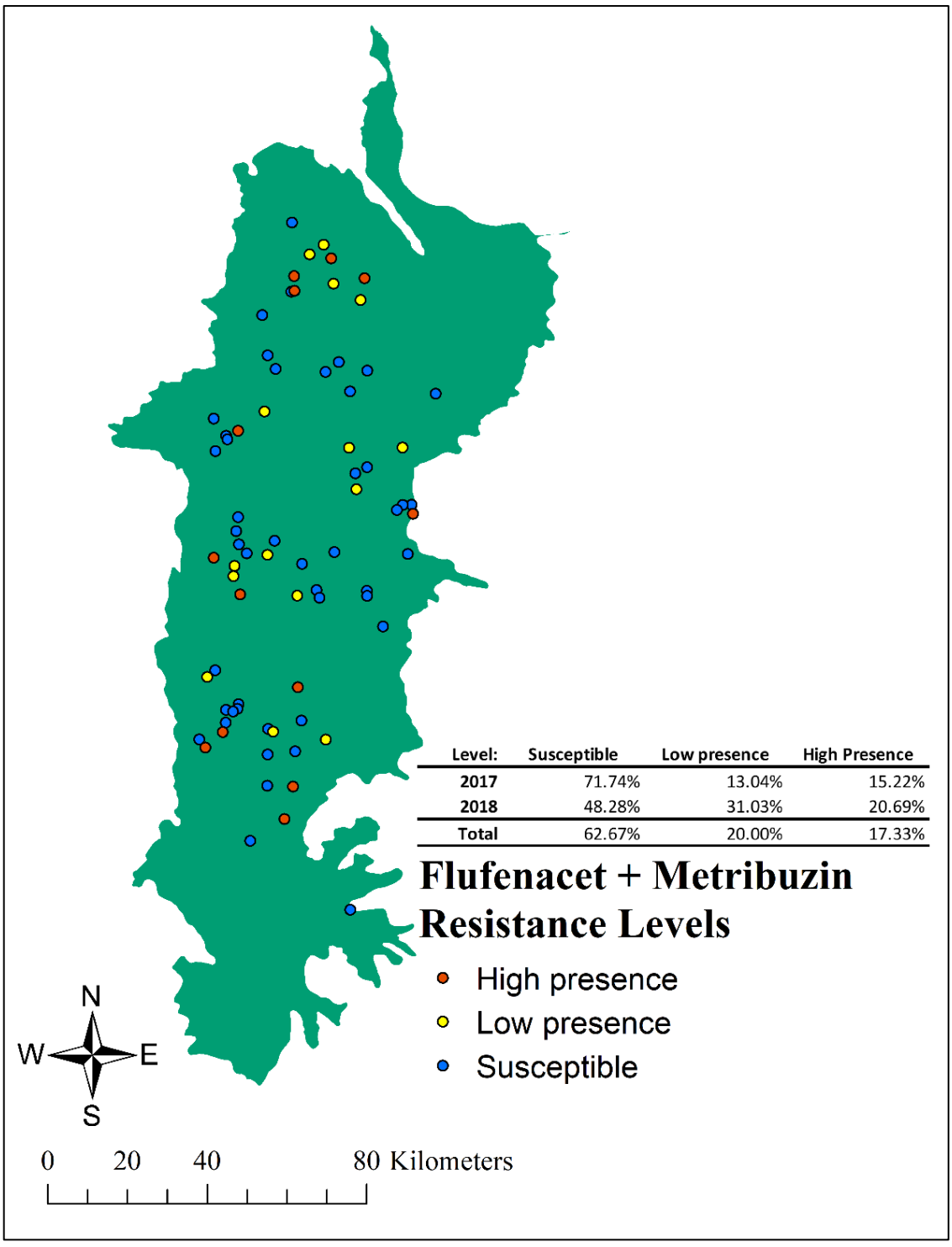



Figure 2-17. Distribution and frequency of resistance to flufenacet + metribuzin in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

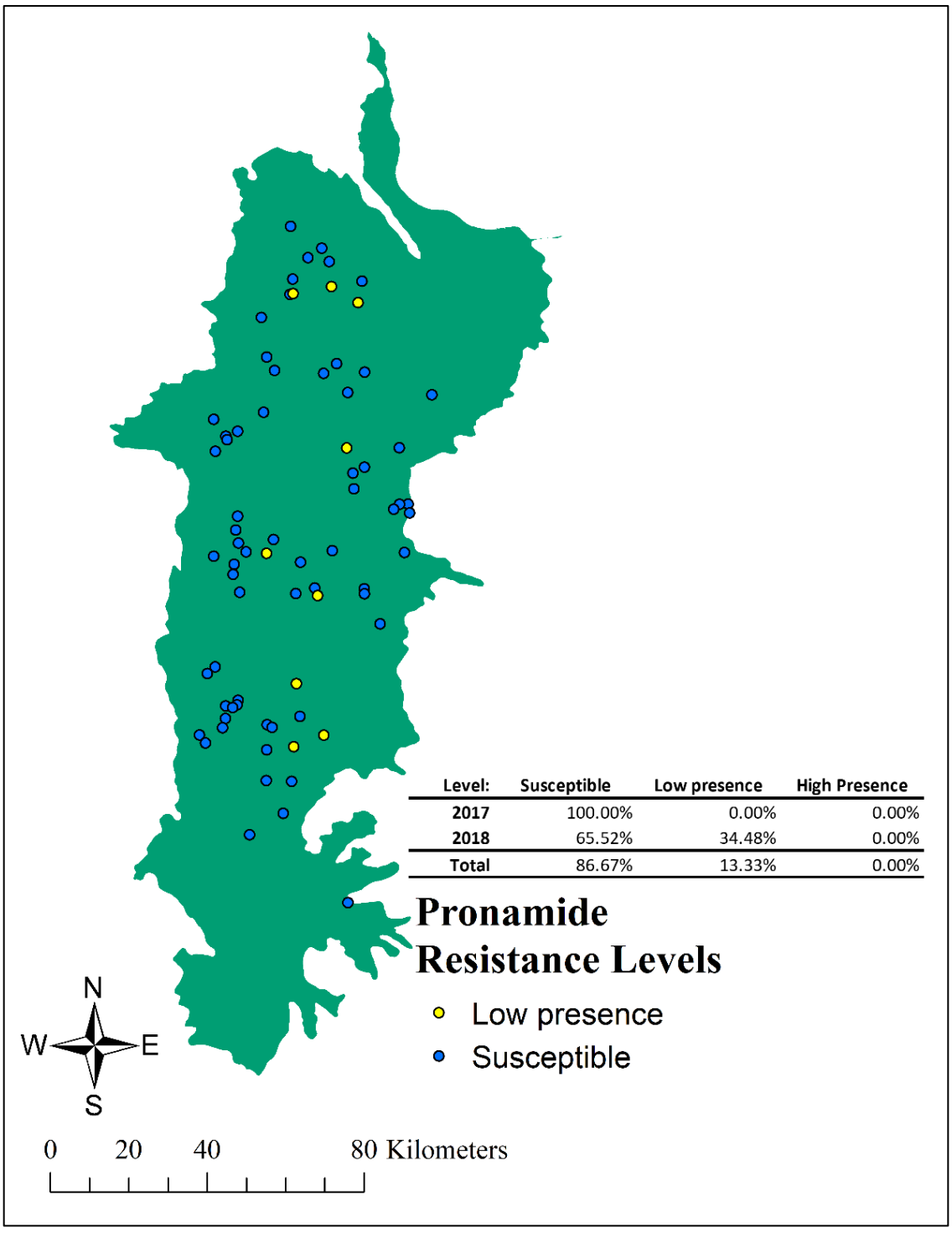



Figure 2-18. Distribution and frequency of resistance to pronamide in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

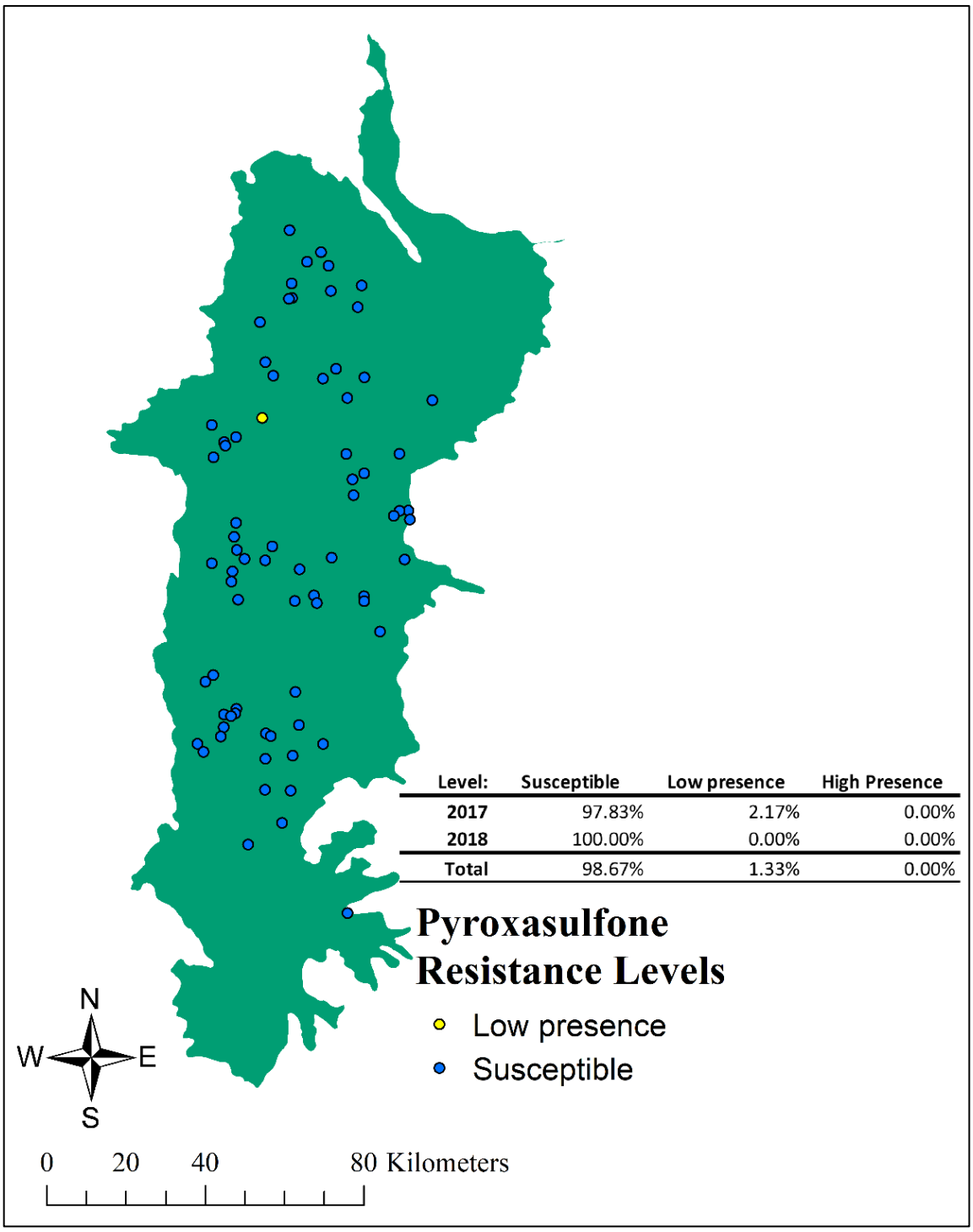



Figure 2-19. Distribution and frequency of resistance to pyroxasulfone in the Willamette Valley, OR. High presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction at least 90%).

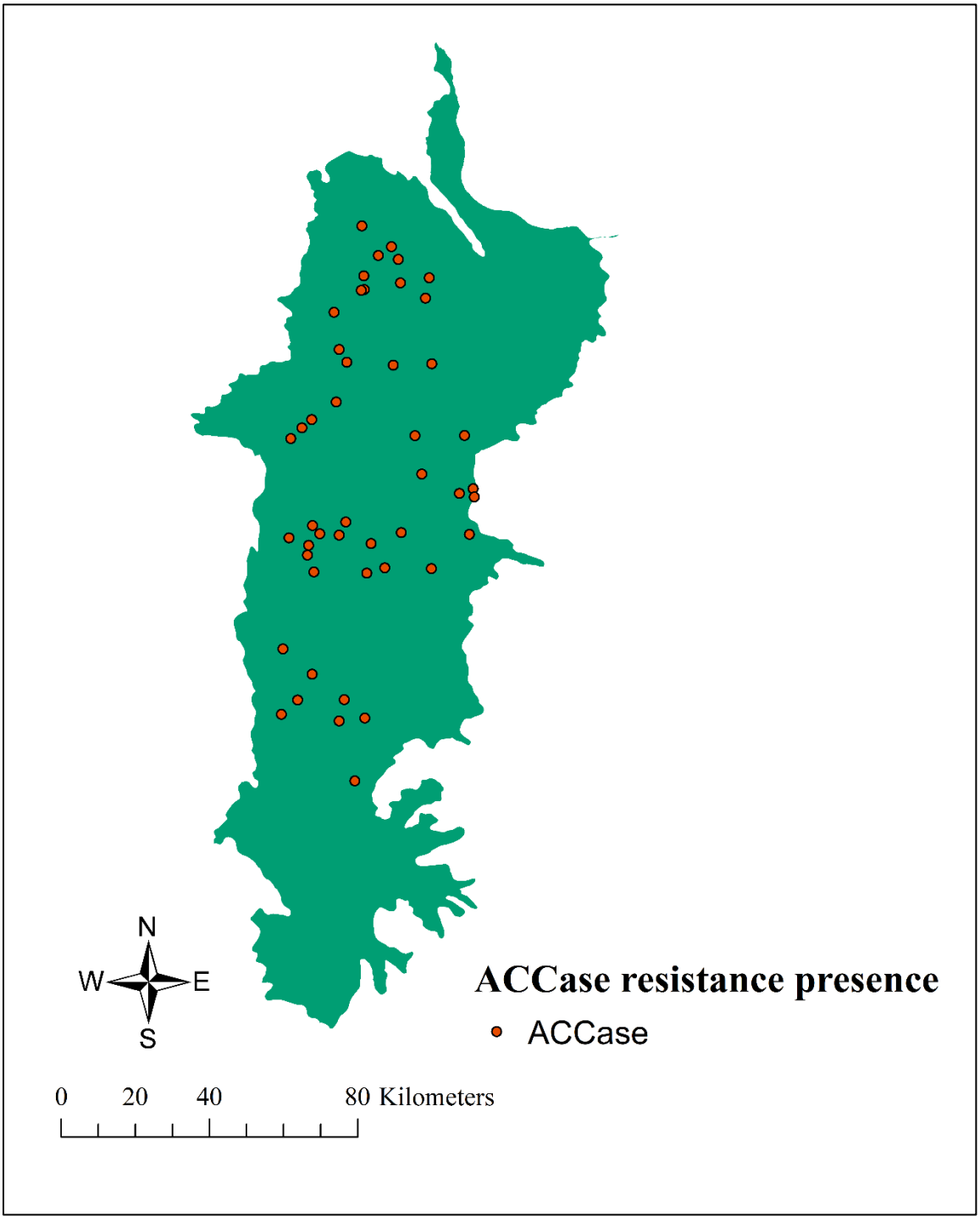



Figure 2-20. Distribution of resistance presence to Acetyl-CoA carboxylase (ACCase) inhibitors in the Willamette Valley, OR. Orange points represent populations carrying the trait.

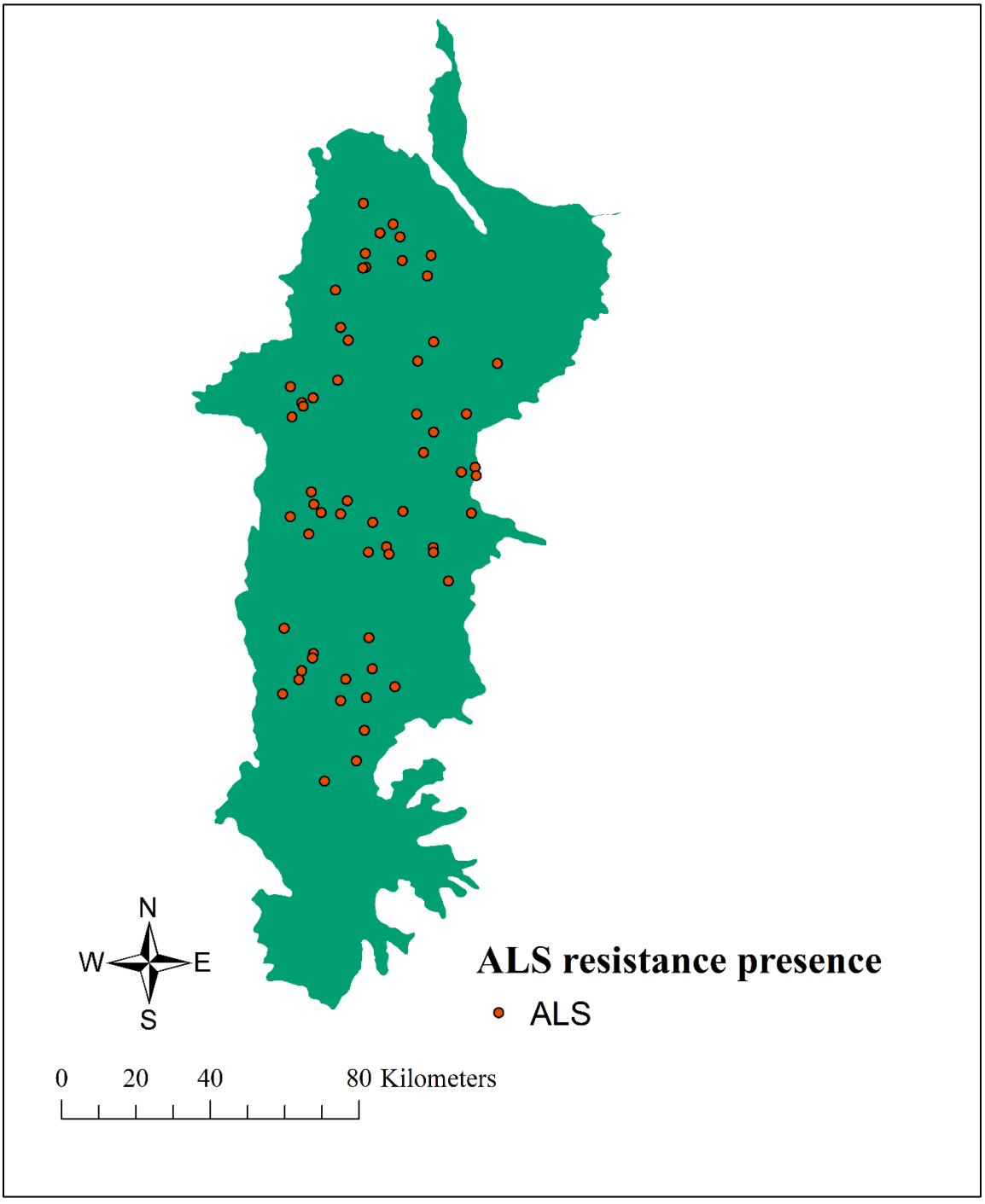



Figure 2-21. Distribution of resistance presence to Acetolactate synthase (ALS) inhibitors in the Willamette Valley, OR. Orange points represent populations carrying the trait.

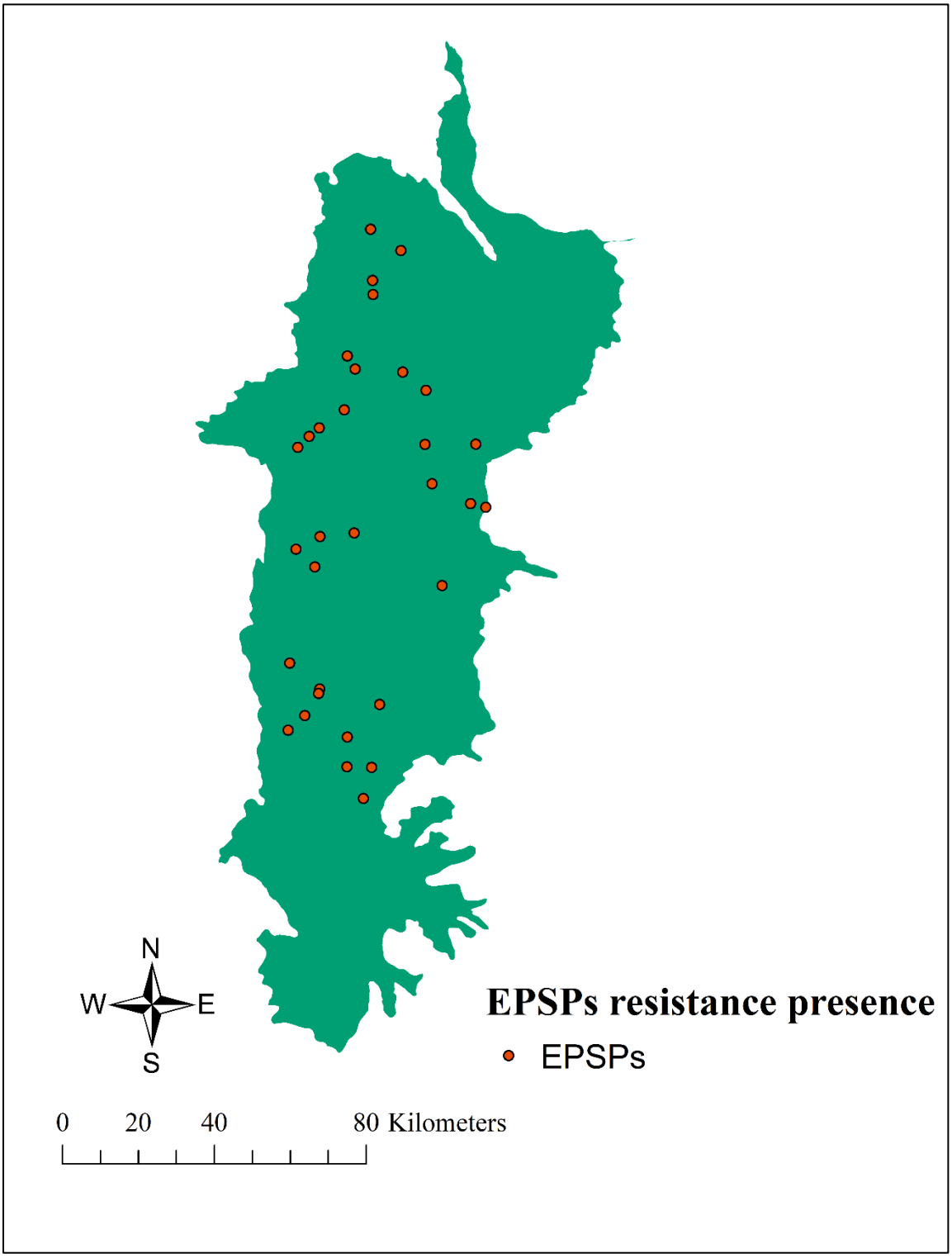



Figure 2-22. Distribution of resistance to 5-enolpyruvylshikimate-3-phosphate (EPSPs) inhibitor in the Willamette Valley, OR. Orange points represent populations carrying the trait.

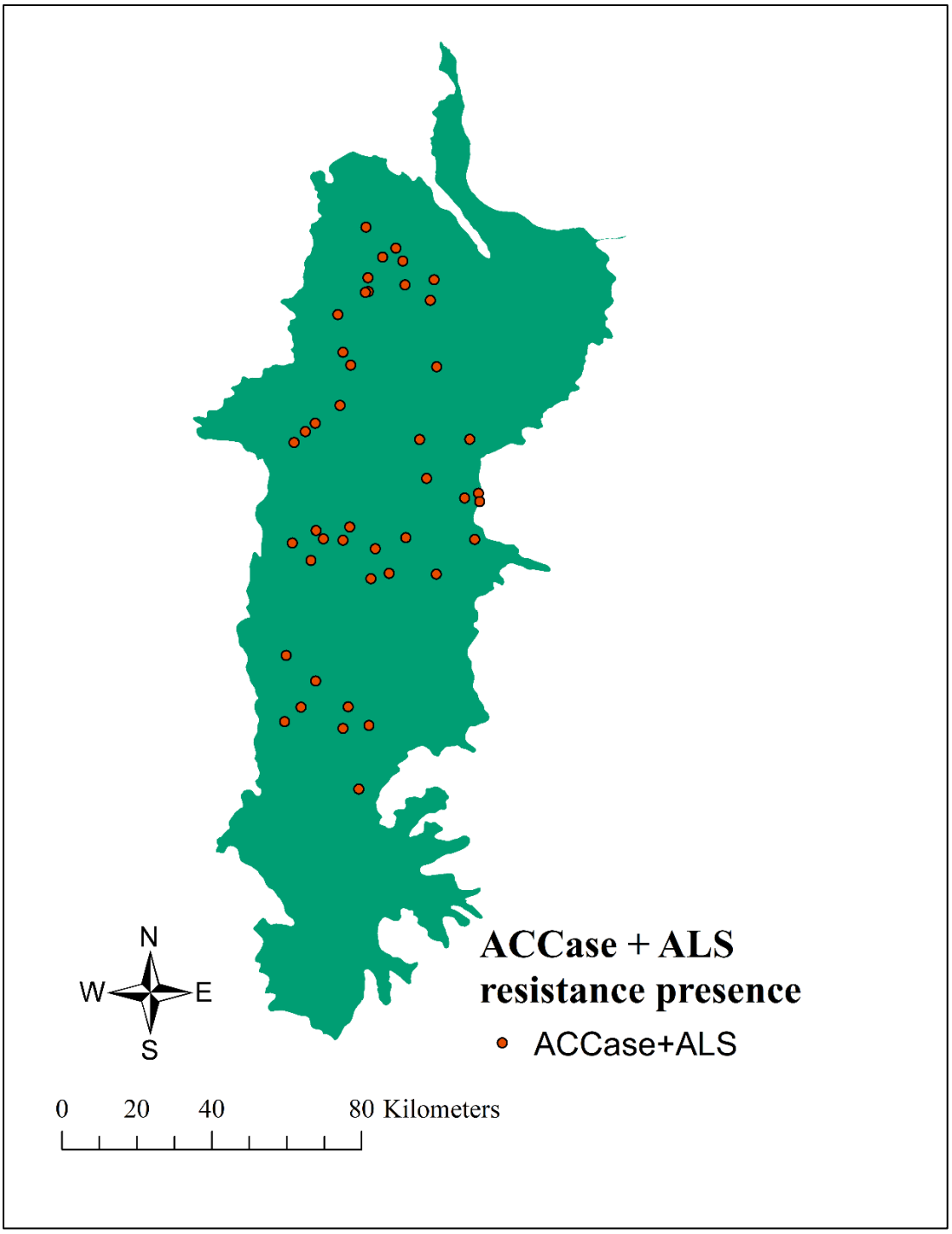



Figure 2-23. Distribution of resistance presence to the combination of Acetyl-CoA carboxylase (ACCase) and acetolactate synthase (ALS) inhibitor in the Willamette Valley, OR. Orange points represent populations carrying the trait.

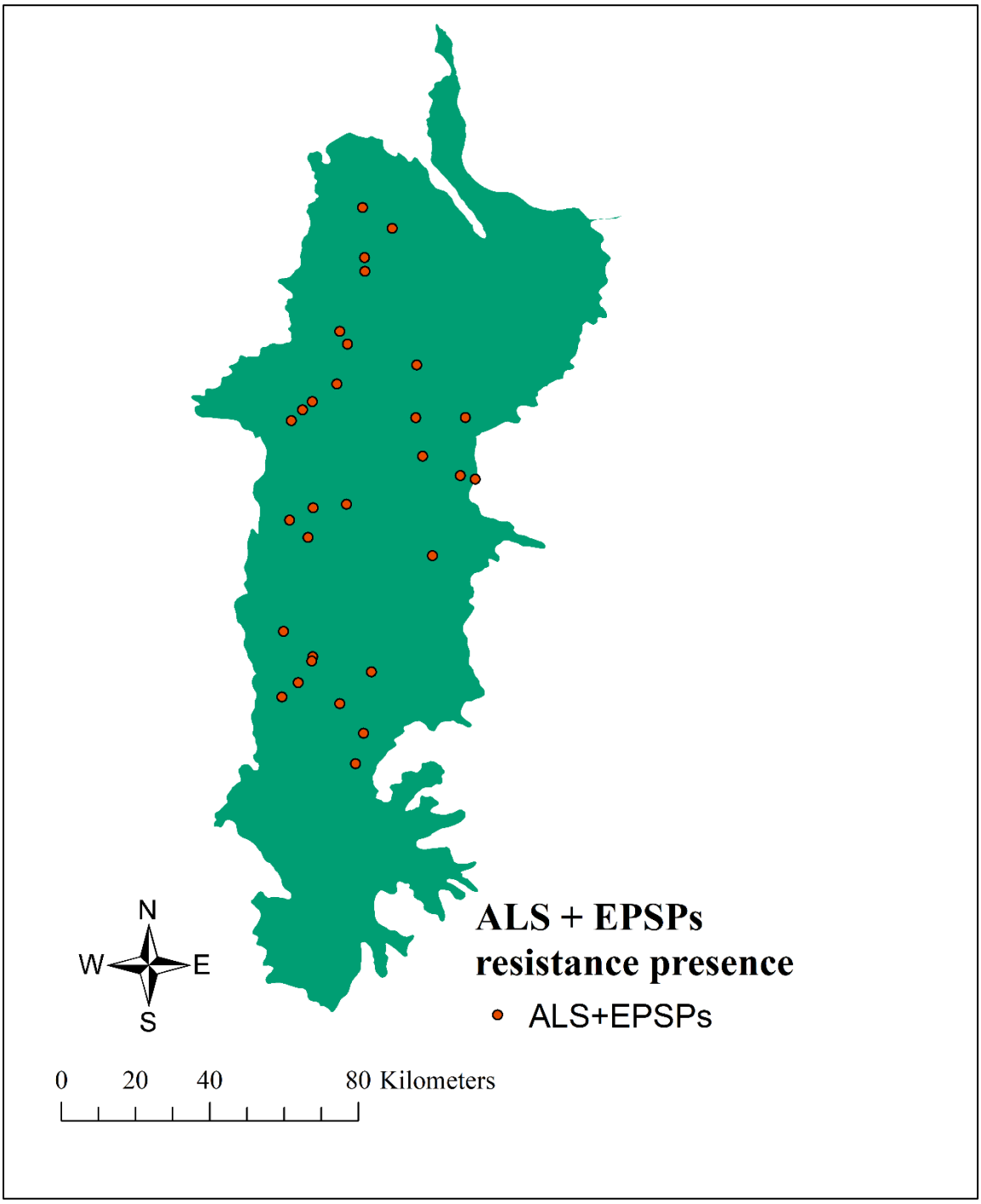



Figure 2-24. Distribution and Frequency of multiple resistance presence to the combination of 5-enolpyruvylshikimate-3-phosphate (EPSPs) and acetolactate synthase (ALS) inhibitor in the Willamette Valley, OR. Orange points represent populations carrying the trait.

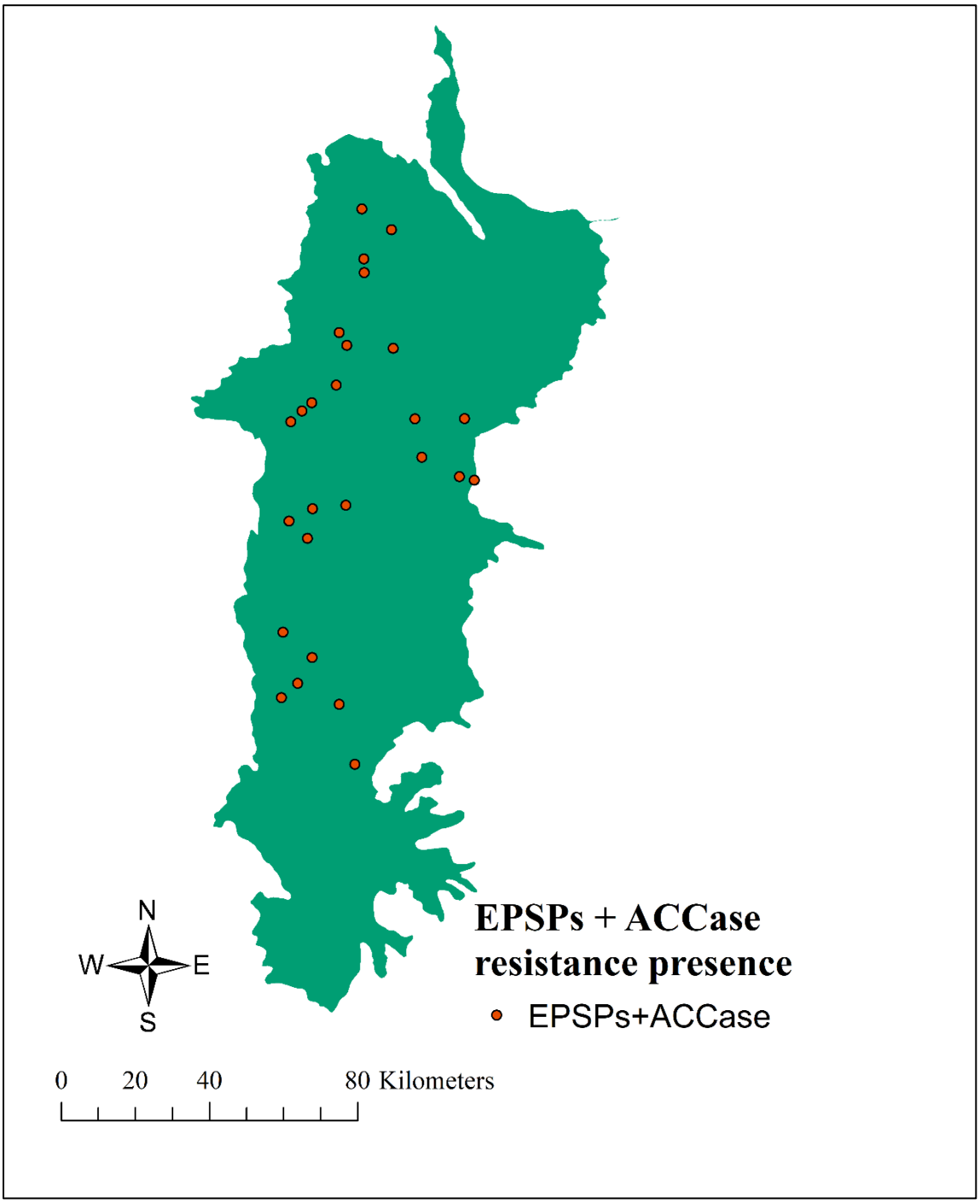



Figure 2-25. Distribution and Frequency of multiple resistance presence to the combination of 5-enolpyruvylshikimate-3-phosphate (EPSPs) and Acetyl-CoA carboxylase (ACCase) inhibitor in the Willamette Valley, OR. Orange points represent populations carrying the trait.

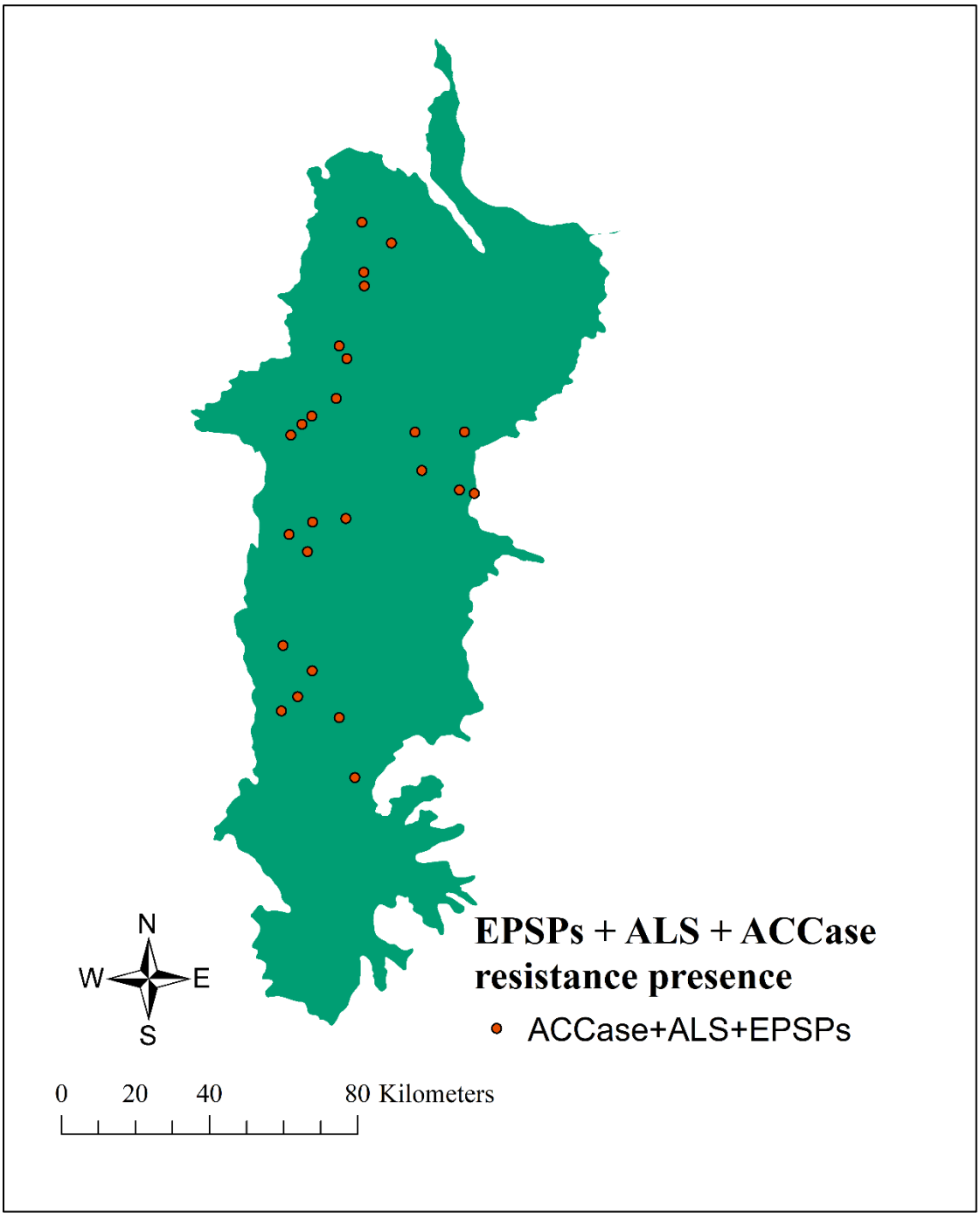



Figure 2-26. Distribution and Frequency of multiple resistance presence to the combination of 5-enolpyruvylshikimate-3-phosphate (EPSPs), acetolactate synthase (ALS) and Acetyl-CoA carboxylase (ACCase) inhibitor in the Willamette Valley, OR. Orange points represent populations carrying the trait.

1

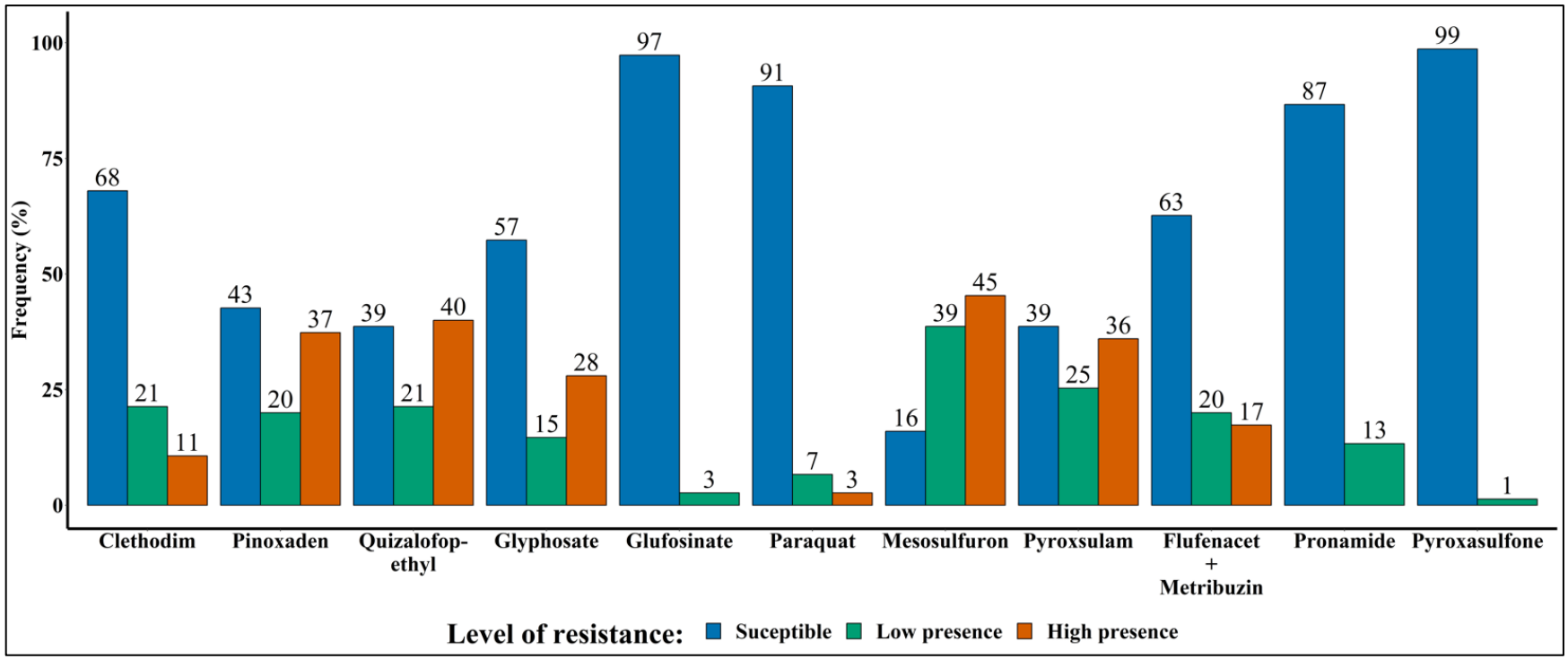



Figure 2-27. Resistance level frequency of populations for each herbicide tested on the two-year survey in the Willamette valley. Where high presence (20% or more survival and less than 70% green area reduction), low presence (2-19% survival and green area reduction between 70 to 90%) and susceptible (less than 2% survival and green area reduction of at least 90%).

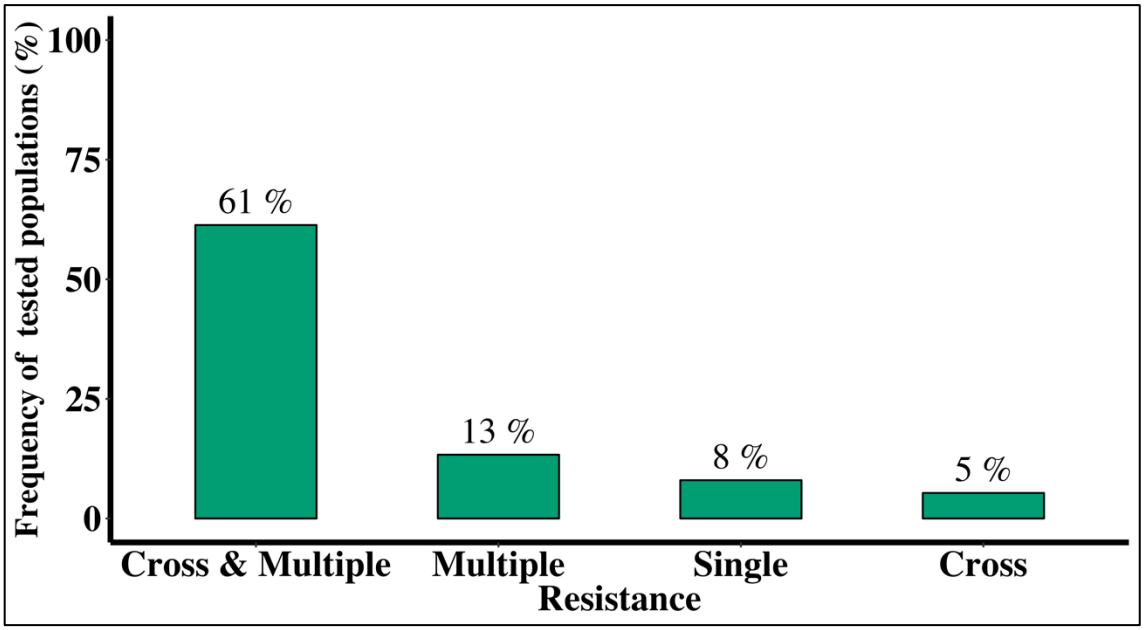



Figure 2-28. Resistance frequency of tested populations of Italian ryegrass in the Willamette Valley.

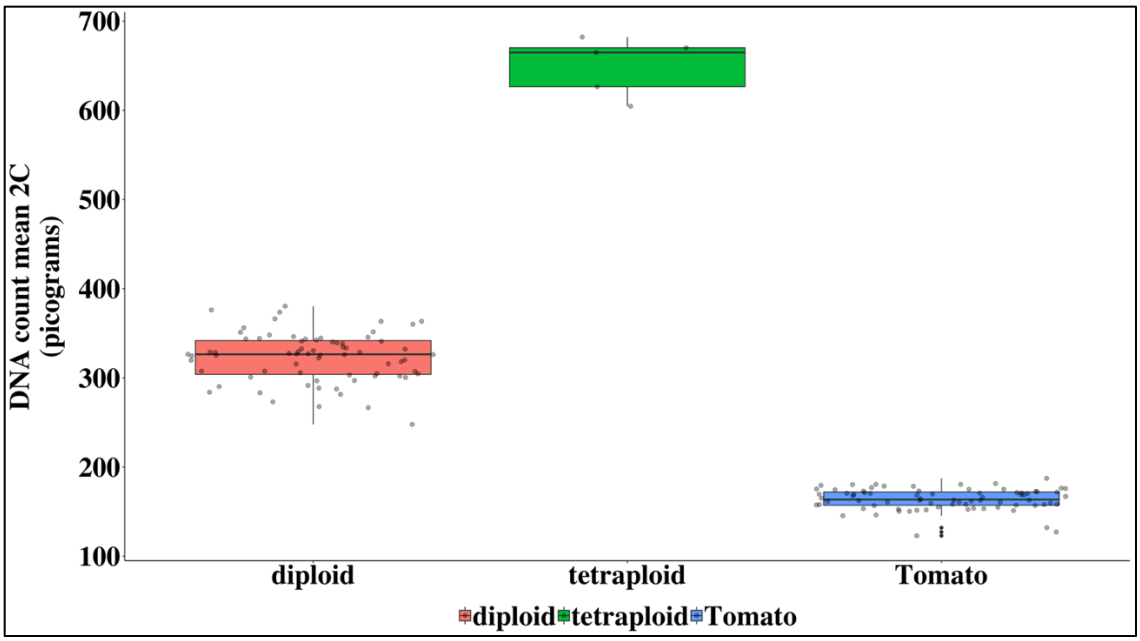



Figure 2-29. Haploid relative genome size (2C) mean results from flow cytometry between the two cultivars of Italian ryegrass and tomato. Diploids (2x = 2n = 14) and tetraploids (2x = 4n = 28).

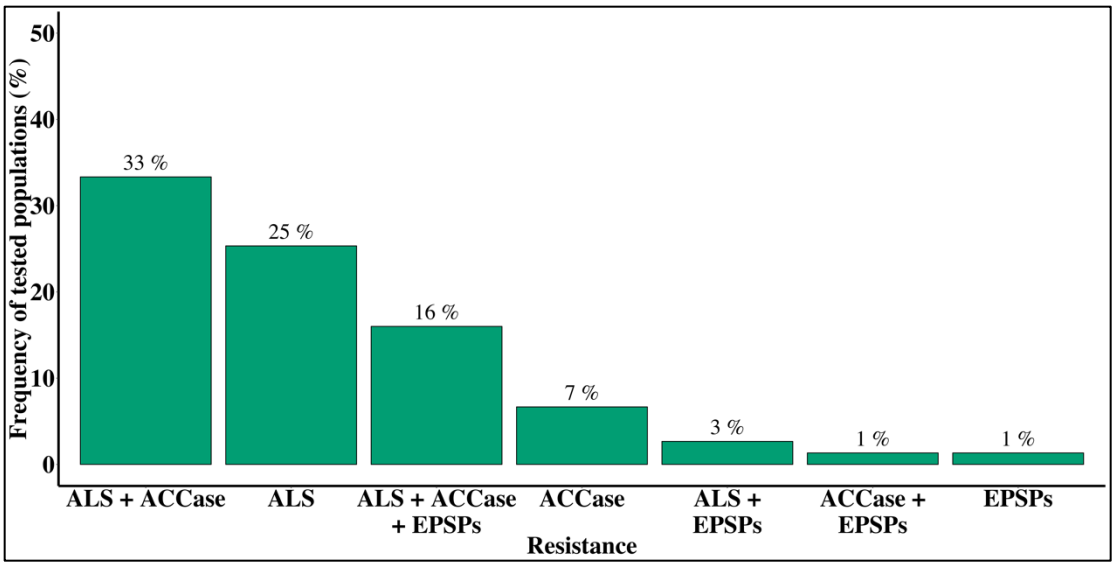



Figure 2-30. Frequency of populations for the most common mode of actions and their combinations of resistance.

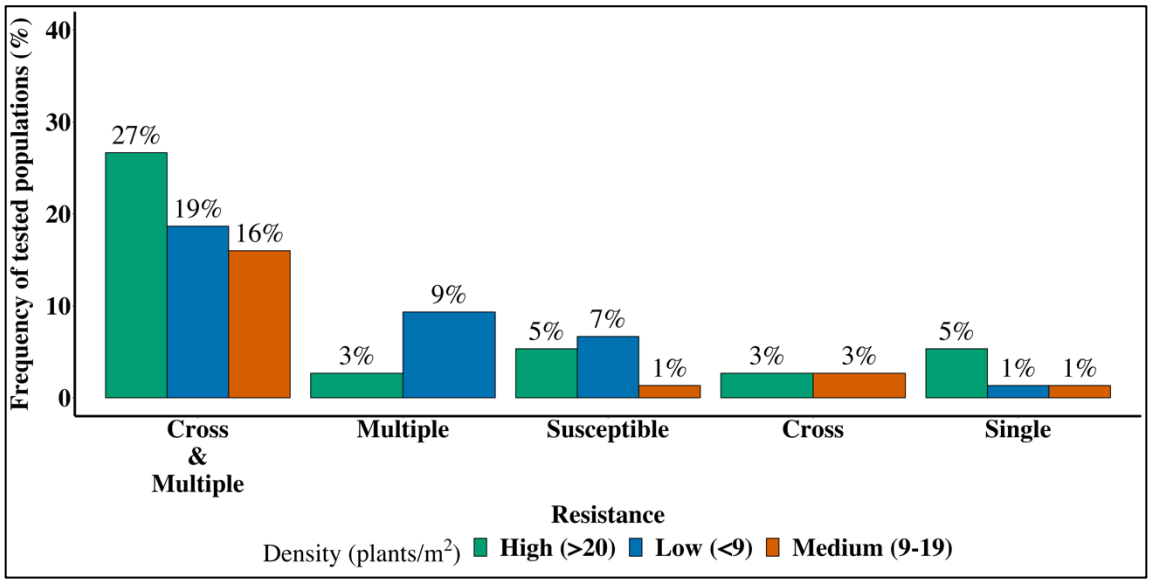



Figure 2-31. Frequency of populations for the types of resistance according to the density level of Italian ryegrass infestation.

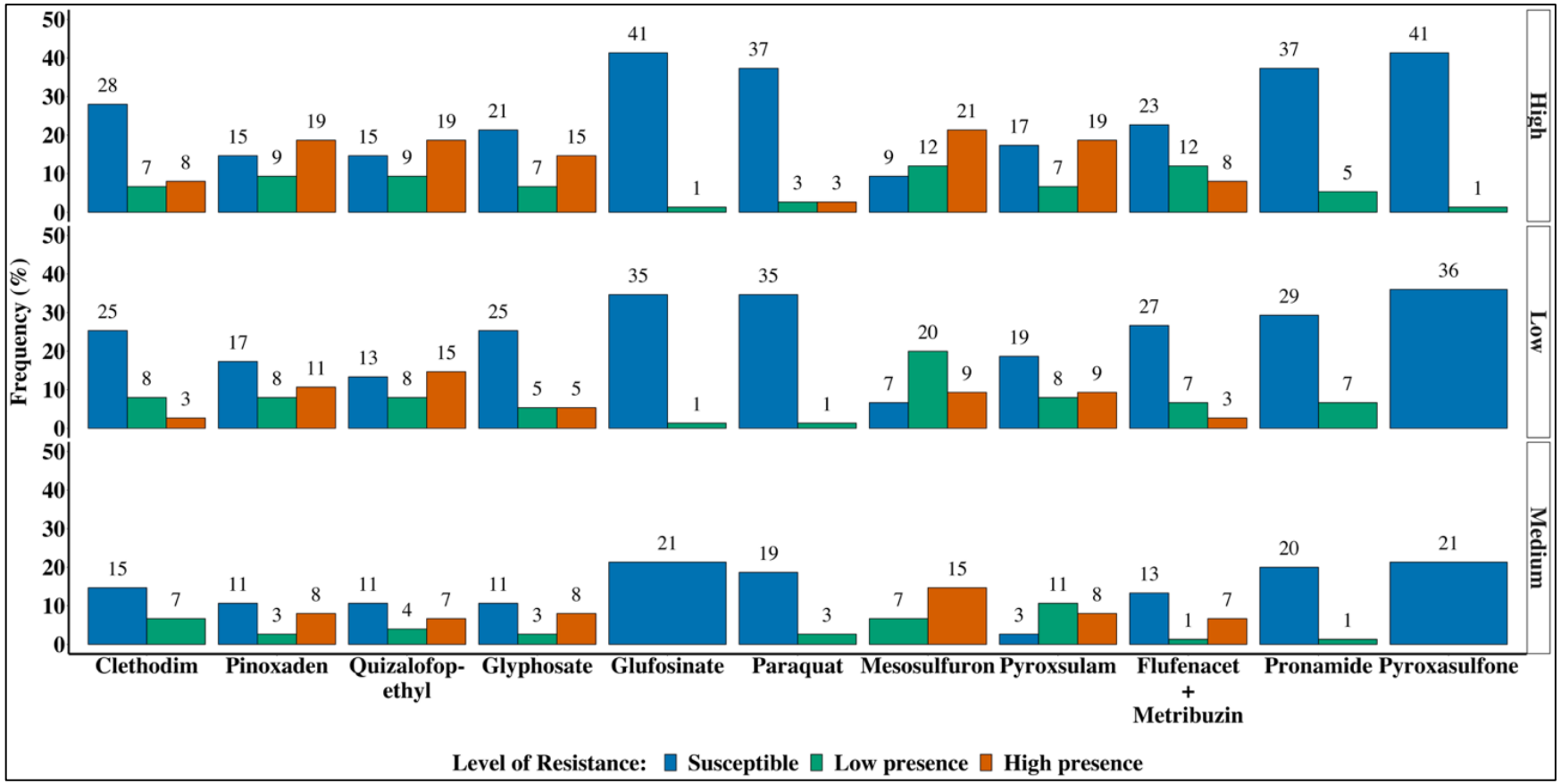



Figure 2-32. Frequency of level of resistance to each herbicide tested according to the density of plants/$m^2$ per populations (High: 20 >=; Low: 9 <; Medium: 9 > and < 20). High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

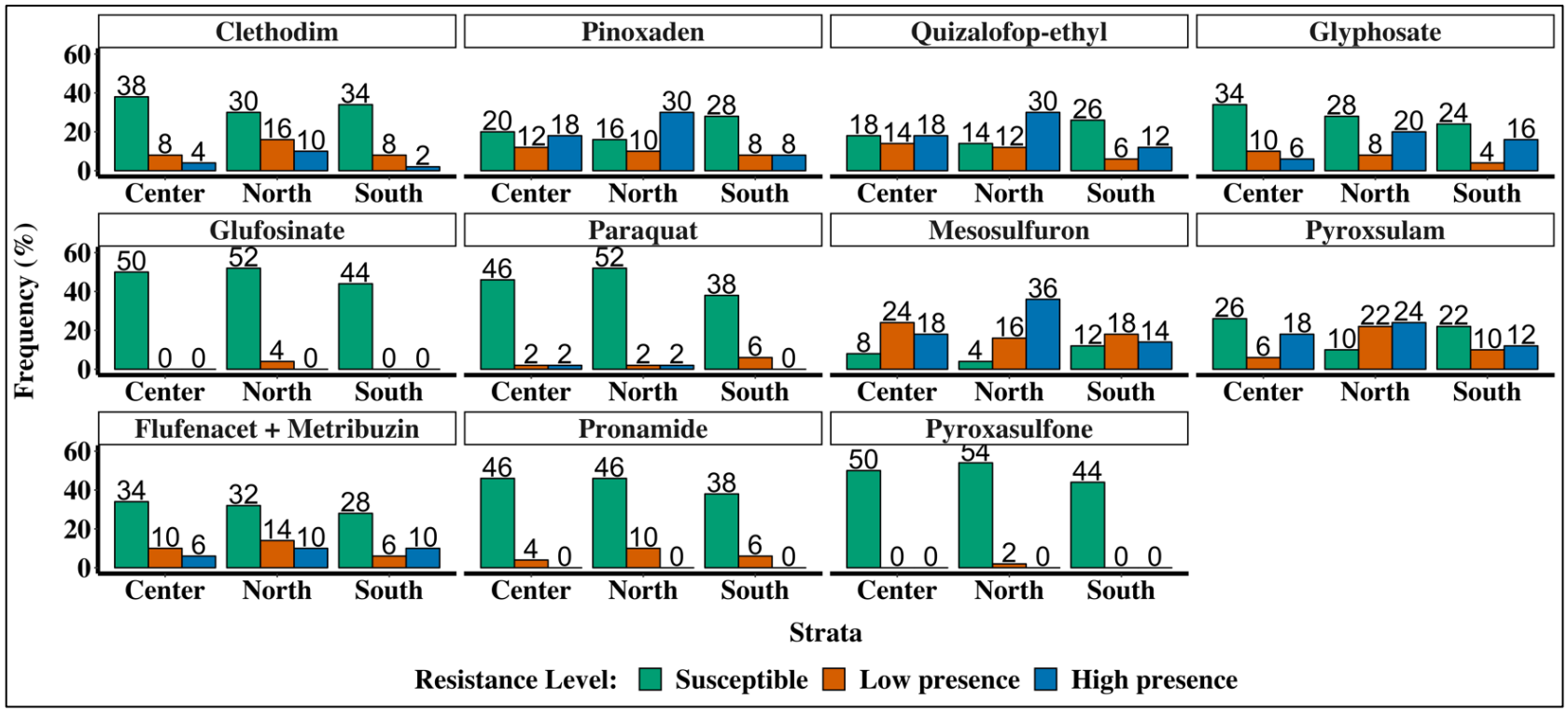



Figure 2-33. Frequency of populations for the resistance levels to each tested herbicide according to the Stratum. Where high presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%)

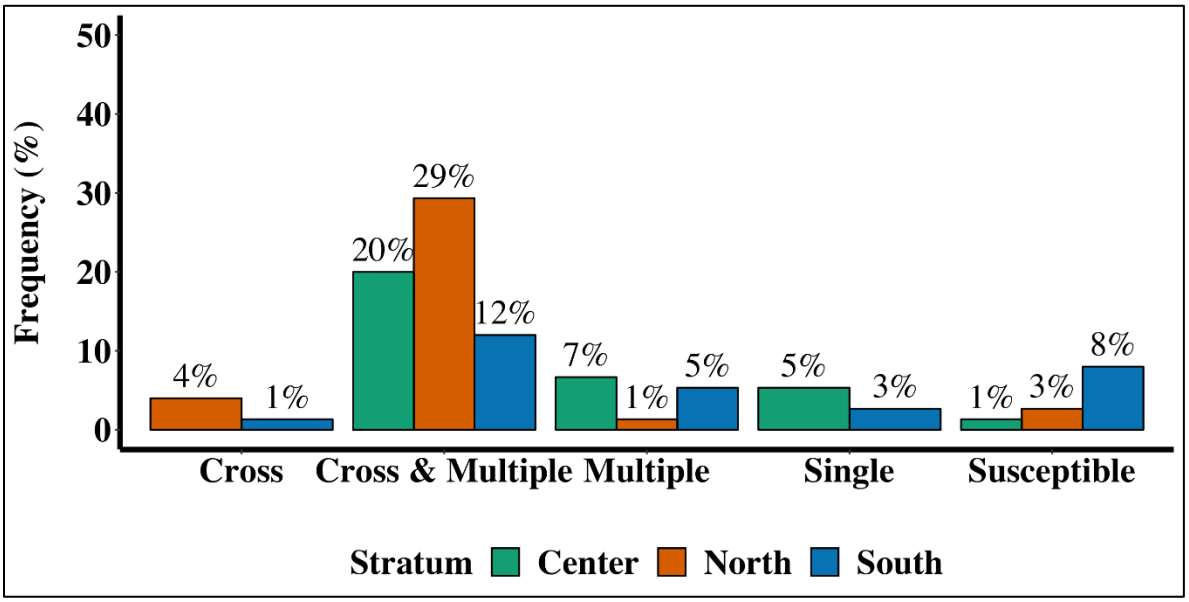



Figure 2-34. Frequency of populations for each resistance type per Stratum of the surveyed area.

# Tables

Table 2-1. Post-emergent herbicides and rates used for herbicide resistance screening test.

| WSSA Group | Active ingredient | Mode of action | Trade name | Product (g/ha) | Rate (g a.i/ha) |
|---|---|---|---|---|---|
| 1 | Quizalofop-P-ethyl | ACCase | Assure II | 840.64 | 92.50† |
| 1 | Clethodim | ACCase | SelectMAX | 1,120.85 | 136.00† |
| 1 | Pinoxaden | ACCase | Axial XL | 1,148.87 | 60.30† |
| 2 | Pyroxsulam | ALS | Powerflex HL | 140.11 | 18.40§ |
| 2 | Mesosulfuron-Methyl | ALS | Osprey | 332.75 | 14.97§ |
| 9 | Glyphosate | EPSPs | Makaze | 2,241.70 | 840.00† |
| 10 | Glufosinate | Glutamine synthase | Rely 280 | 4,203.18 | 1,150.00† |
| 22 | Paraquat | PS I | Gramoxone SL 2.0 | 280.21 | 1,120.00† |

†: NIS 0.25 %V/V; §: NIS 0.25 %V/V + AMS 3.36 kg/ha

Table 2-2. Pre-emergent herbicides and rates used for herbicide resistance screening test.

| WSSA Group | Active ingredient | Mode of action | Trade name | Rate (μM) |
|---|---|---|---|---|
| 15 + 5 | Flufenacet + Metribuzin | VLFA† + PS II § | Axiom | 2.20 |
| 15 | Pyroxasulfone | VLFA† | Zidua | 0.98 |
| 5 | Pronamide | Microtubule assembly | Kerb | 1.40 |

†: Very long fatty-acid inhibitor; §: Photosystem II inhibitor

Table 2-3. Frequency of different modes of action with resistance combinations on tested populations of Italian ryegrass.

| Modes of action resistance | Number of cases | Frequency (%) |
|---|---|---|
| ACCase+ALS | 10 | 13% |
| ACCase+ALS+Mitotic | 1 | 1% |
| ACCase+ALS+VLFA | 5 | 7% |
| ACCase+ALS+VLFA+Mitotic | 3 | 4% |
| ACCase+EPSPs | 1 | 1% |
| ACCase+EPSPs+ALS | 9 | 12% |
| ACCase+EPSPs+ALS+PS1 | 1 | 1% |
| ACCase+EPSPs+ALS+VLFA | 10 | 13% |
| ACCase+EPSPs+ALS+VLFA+Mitotic | 2 | 3% |
| ACCase+EPSPs+ALS+VLFA+PS1 | 3 | 4% |
| ACCase+VLFA | 2 | 3% |
| ALS | 8 | 11% |
| ALS+Mitotic | 1 | 1% |
| ALS+PS1 | 1 | 1% |
| ALS+VLFA+Mitotic | 1 | 1% |
| ALS+VLFA+PS1+Mitotic | 1 | 1% |
| EPSPs | 1 | 1% |
| EPSPs+ALS | 4 | 5% |
| EPSPs+ALS+VLFA | 1 | 1% |
| PS1 | 1 | 1% |
| Susceptible | 9 | 12% |

Table 2-4. Frequency of presence of Italian ryegrass in the surveyed fields per stratum.

| | Not present | Present | Total | Stratum | Survey |
|---|---|---|---|---|---|
| **Stratum** | **Number of fields** | | | **Frequency (%)** | |
| Center | 25 | 25 | 50 | 50 | 17 |
| North | 22 | 28 | 50 | 56 | 19 |
| South | 28 | 22 | 50 | 44 | 15 |
| **Total** | **75** | **75** | **150** | **50** | **50** |

Table 2-5. Frequency of presence of Italian ryegrass in the surveyed fields per crop.

| | Not present | Present | Total | Crop | Survey |
|---|---|---|---|---|---|
| **Crop** | **Number of fields** | | | **Frequency (%)** | |
| Oat | 1 | 1 | 2 | 50 | 1 |
| Orchard grass | 3 | 10 | 13 | 77 | 7 |
| Tall fescue | 59 | 40 | 99 | 40 | 27 |
| Tree crop | 1 | 5 | 6 | 83 | 3 |
| Wheat | 8 | 18 | 26 | 69 | 12 |
| Clover | 3 | 1 | 4 | 25 | 1 |
| **Total** | **75** | **75** | **150** | **50** | **50** |

Table 2-6. Frequency of density per stratum in the survey area.

| | Stratum | | |
|---|---|---|---|
| **Density** | **Center** | **North** | **South** |
| **Plants/m²†** | **Frequency (%)** | | |
| **High** | 12 | 16 | 15 |
| **Low** | 15 | 12 | 9 |
| **Medium** | 7 | 9 | 5 |

†: high (20≥ plants/m²), medium (10 to 19 plants/m²) and low (1 to 9 plants/m²).

Table 2-7. Frequency of resistance to all tested herbicide and number of fields surveyed for tall fescue crop.

| **Crop:** | **Tall Fescue** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **High presence†** | | | **Low presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 31 | 40 | 78 | 0 | 40 | 0 | 9 | 40 | 23 |
| Pinoxaden | 21 | 40 | 53 | 11 | 40 | 28 | 8 | 40 | 20 |
| Quizalofop-p-ethyl | 17 | 40 | 43 | 16 | 40 | 40 | 7 | 40 | 18 |
| Glyphosate | 25 | 40 | 63 | 10 | 40 | 25 | 5 | 40 | 13 |
| Glufosinate | 40 | 40 | 100 | 0 | 40 | 0 | 0 | 40 | 0 |
| Paraquat | 37 | 40 | 93 | 0 | 40 | 0 | 3 | 40 | 8 |
| Mesosulfuron | 9 | 40 | 23 | 13 | 40 | 33 | 18 | 40 | 45 |
| Pyroxsulam | 20 | 40 | 50 | 7 | 40 | 18 | 13 | 40 | 33 |
| Flufenacet + Metribuzin | 25 | 40 | 63 | 8 | 40 | 20 | 7 | 40 | 18 |
| Pronamide | 36 | 40 | 90 | 0 | 40 | 0 | 4 | 40 | 10 |
| Pyroxasulfone | 40 | 40 | 100 | 0 | 40 | 0 | 0 | 40 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table 2-8. Frequency of resistance to all tested herbicide and number of fields surveyed for wheat crop.

| Crop: | Wheat | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **High presence†** | | | **Low presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 8 | 18 | 44 | 5 | 18 | 28 | 5 | 18 | 28 |
| Pinoxaden | 2 | 18 | 11 | 13 | 18 | 72 | 3 | 18 | 17 |
| Quizalofop-p-ethyl | 4 | 18 | 22 | 11 | 18 | 61 | 3 | 18 | 17 |
| Glyphosate | 10 | 18 | 56 | 4 | 18 | 22 | 4 | 18 | 22 |
| Glufosinate | 18 | 18 | 100 | 0 | 18 | 0 | 0 | 18 | 0 |
| Paraquat | 17 | 18 | 94 | 0 | 18 | 0 | 1 | 18 | 6 |
| Mesosulfuron | 0 | 18 | 0 | 15 | 18 | 83 | 3 | 18 | 17 |
| Pyroxsulam | 2 | 18 | 11 | 15 | 18 | 83 | 1 | 18 | 6 |
| Flufenacet + Metribuzin | 9 | 18 | 50 | 3 | 18 | 17 | 6 | 18 | 33 |
| Pronamide | 15 | 18 | 83 | 0 | 18 | 0 | 3 | 18 | 17 |
| Pyroxasulfone | 17 | 18 | 94 | 0 | 18 | 0 | 1 | 18 | 6 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table 2-9 Frequency of type of resistance per crop on tall fescue and wheat found on the surveyed fields.

| Crop | Type of resistance | Fields | Total | Frequency (%) |
|---|---|---|---|---|
| Tall fescue | Susceptible | 8 | 40 | 20 |
| | Single | 4 | 40 | 10 |
| | Multiple | 6 | 40 | 15 |
| | Cross | 2 | 40 | 5 |
| | Cross & Multiple | 20 | 40 | 50 |
| Wheat | Susceptible | 1 | 18 | 6 |
| | Single | - | 18 | - |
| | Multiple | - | 18 | - |
| | Cross | 1 | 18 | 6 |
| | Cross & Multiple | 16 | 18 | 88 |

Table 2-10 Frequency of type of resistance in tree crop, clover and oat in the surveyed fields.

| Crop | Type of resistance | Fields | Total | Frequency (%) |
|---|---|---|---|---|
| Tree crop | Susceptible | - | - | - |
| | Single | 1 | 5 | 20 |
| | Multiple | 2 | 5 | 40 |
| | Cross | - | - | - |
| | Cross & Multiple | 2 | 5 | 40 |
| Clover | Susceptible | - | - | - |
| | Single | - | - | - |
| | Multiple | - | - | - |
| | Cross | - | - | - |
| | Cross & Multiple | 1 | 1 | 100 |
| Oat | Susceptible | - | - | - |
| | Single | - | - | - |
| | Multiple | - | - | - |
| | Cross | - | - | - |
| | Cross & Multiple | 1 | 1 | 100 |

Table 2-11. Results for Italian ryegrass presence of binomial logit analysis

| | Ryegrass Presence | | Confidence Interval | | |
|---|---|---|---|---|---|
| | **Comparison** | **Odds-Ratio** | **Lower** | **Upper** | **p-value** |
| | Intercept | 0.747 | 0.351 | 1.569 | 0.444 |
| **Elevation** | Elevation | 1.000 | 0.994 | 1.007 | 0.897 |
| **Crop** | Tall Fescue vs Wheat | 3.121 | 1.217 | 8.604 | *0.021 |
| | Tall Fescue vs Tree crop | 7.127 | 1.081 | 140.031 | 0.079 |
| | Tall Fescue vs Orchardgrass | 4.928 | 1.393 | 23.166 | *0.022 |
| | Tall Fescue vs Clover | 0.500 | 0.024 | 4.080 | 0.555 |
| | Tall Fescue vs Oat | 1.524 | 0.057 | 40.924 | 0.773 |
| **Location** | North vs Center | 0.850 | 0.323 | 2.226 | 0.740 |
| | North vs South | 0.810 | 0.338 | 1.943 | 0.635 |

| | Wald Test for overall effect of crop type | | | |
|---|---|---|---|---|
| **Response** | **Variable** | **Chi-Square** | **DF** | **p-value** |
| Ryegrass Presence | Crop | 12.5 | 5 | *0.029 |

* : 0.05 alpha of p-value significance

Table 2-12. Results for herbicide resistance presence of binomial logit analysis

| | Resistance presence | | Confidence Interval | | |
|---|---|---|---|---|---|
| Variable | **Comparison** | **Odds-Ratio** | **Lower** | **Upper** | **p-value** |
| | Intercept | 1.744 | 0.443 | 7.092 | 0.422 |
| Elevation | elevation | 0.999 | 0.989 | 1.009 | 0.769 |
| Crop | Tall Fescue vs Wheat | 3.246 | 0.655 | 24.380 | 0.182 |
| | Tall Fescue vs Tree crop | 0.965 | 0.000 | 46.432 | 0.993 |
| | Tall Fescue vs Orchardgrass | 0.584 | 0.125 | 2.819 | 0.489 |
| | Tall Fescue vs Clover | 0.000 | 0.000 | Inf | 0.997 |
| | Tall Fescue vs Oat | 0.000 | 0.000 | Inf | 0.997 |
| Location | North vs Center | 0.780 | 0.160 | 3.800 | 0.755 |
| | North vs South | 0.428 | 0.091 | 1.885 | 0.266 |
| Density | Low vs Medium | 3.416 | 0.768 | 19.398 | 0.127 |
| | Low vs High | 3.630 | 0.994 | 15.283 | 0.060 |

Table 2-13. Results for multiple herbicide resistance presence (considering only high presence of resistance) of binomial logit analysis

| | Multiple resistance presence | | Confidence Interval | | |
|---|---|---|---|---|---|
| | **Variable** | **Odd-Ratio** | **Lower** | **Upper** | **p-value** |
| | Intercept | 1.163 | 0.278 | 4.718 | 0.832 |
| Elevation | elevation | 1.000 | 0.989 | 1.010 | 0.936 |
| Crop | Tall Fescue vs Wheat | 3.990 | 0.766 | 31.052 | 0.126 |
| | Tall Fescue vs Tree crop | 0.979 | 0.101 | 22.299 | 0.987 |
| | Tall Fescue vs Orchardgrass | 0.257 | 0.046 | 1.218 | 0.097 |
| | Tall Fescue vs Clover | 0.000 | 0.000 | Infinity | 0.995 |
| | Tall Fescue vs Oat | 0.000 | 0.000 | infinity | 0.995 |
| Location | North vs Center | 0.665 | 0.141 | 3.086 | 0.600 |
| | North vs South | 0.323 | 0.069 | 1.376 | 0.133 |
| Density | Low vs Medium | 7.503 | 1.581 | 48.481 | 0.018* |
| | Low vs High | 5.269 | 1.430 | 22.856 | 0.017* |

| Wald Test for overall effect of Density level | | | | |
|---|---|---|---|---|
| **Response** | **Variable** | **Chi-Square** | **DF** | **p-value** |
| Multiple resistance | Density level | 8 | 2 | *0.018 |

*: 0.05 alpha of p-value significance

Table 2-14. Results for cross herbicide resistance presence (considering only high presence of resistance) of binomial logit analysis

| | Cross resistance | | Confidence Interval | | |
|---|---|---|---|---|---|
| | **Variable** | **Odd-Ratio** | **Lower** | **Upper** | **p-value** |
| Elevation | Intercept | 0.367 | 0.083 | 1.346 | 0.151 |
| | elevation | 1.002 | 0.992 | 1.012 | 0.679 |
| Crop | Tall Fescue vs Wheat | 10.342 | 2.491 | 56.068 | 0.003* |
| | Tall Fescue vs Tree crop | 0.550 | 0.025 | 4.959 | 0.628 |
| | Tall Fescue vs Orchardgrass | 1.647 | 0.341 | 7.458 | 0.518 |
| | Tall Fescue vs Clover | 0.000 | 0.000 | infinity | 0.994 |
| | Tall Fescue vs Oat | 0.000 | 0.000 | infinity | 0.994 |
| Location | North vs Center | 0.542 | 0.121 | 2.326 | 0.410 |
| | North vs South | 0.458 | 0.108 | 1.891 | 0.280 |
| Density | Low vs Medium | 2.587 | 0.618 | 11.377 | 0.196 |
| | Low vs High | 1.326 | 0.351 | 4.958 | 0.672 |

**Wald Test for overall effect of crop type**

| **Response** | **Variable** | **Chi-Square** | **DF** | **p-value** |
|---|---|---|---|---|
| Cross resistance | Crop | 10.5 | 5 | 0.072 |

*: 0.05 alpha of p-value significance

Table 2-15. Nearest neighbor cluster analysis results for types of resistance and the most frequents MOA (modes of action) resistance

| | Type of resistance | | | Most frequent MOA resistance | | |
|---|---|---|---|---|---|---|
| | **Resistance** | **Multiple-Resistance** | **Cross-Resistance** | **ACCase** | **EPSPs** | **ALS** |
| **Euclidian distance unit:** | ----------*km*---------- | | | | | |
| **Observed Mean Distance:** | 5.75 | 5.89 | 6.41 | 3.86 | 5.07 | 5.01 |
| **Expected Mean Distance:** | 6.01 | 7.07 | 7.08 | 4.18 | 5.58 | 5.18 |
| **Nearest Neighbor Ratio:** | 0.96 | 0.83 | 0.90 | 0.92 | 0.91 | 0.97 |
| ***z*-score:** | -0.62 | -1.88 | -1.05 | -0.88 | -0.84 | -0.33 |
| ***p*-value:** | 0.54 | 0.06* | 0.30 | 0.38 | 0.40 | 0.74 |
| **Pattern:** | **random** | **clustered** | **random** | **random** | **random** | **random** |

* : 0.10 alpha level of p-value significance

Table 2-16. Nearest neighbor cluster analysis results for the high presence of resistance level on the tested herbicides that presented this level

| | Resistance level: High presence | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Cleth.†** | **Pinox. †** | **Qui†** | **Glyph†** | **Paraq†** | **Pyroxs†** | **Mesos†.** | **Fluf† + Metr†** |
| **Euclidian distance:** | ----------*km*---------- | | | | | | | |
| **Observed Mean:** | 19.00 | 6.71 | 6.50 | 8.11 | 5.75 | 8.02 | 6.08 | 9.48 |
| **Expected Mean:** | 10.15 | 7.30 | 7.42 | 8.92 | 3.37 | 8.29 | 7.30 | 9.26 |
| **Ratio:** | 1.87 | 0.92 | 0.88 | 0.91 | 1.70 | 0.97 | 0.83 | 1.02 |
| **z-score:** | 5.01 | -0.86 | -1.30 | -0.84 | 9.99 | -0.33 | -0.33 | 0.19 |
| **p-value:** | < 0.001 | 0.39 | 0.19 | 0.40 | *< 0.001 | 0.74 | **0.06 | 0.85 |
| **Pattern:** | **Dispersed** | **Random** | **Random** | **Random** | **Dispersed** | **Random** | **Clustered** | **Random** |

* : 0.05 alpha of p-value significance; **: 0.10 alpha of p-value significance / †Cleth = Clethodim; Pinox = Pinoxaden; Quiz = Quizalofop-p-ethyl; Glyph = Glyphosate; Paraq = Paraquat; Mesos= Mesosulfuron; Fluf = Flufenacet; Metr = Metribuzin;

Table 2-17. Nearest neighbor cluster analysis results for the low presence of resistance level on the tested herbicides that presented this level

| | **Resistance level: Low presence** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Cleth.†** | **Pinox.†** | **Quiz†.** | **Glyph.†** | **Paraq.†** | **Pyroxs†** | **Mesos.†** | **Fluf.† + Metr.†** | **Pron.†** |
| **Euclidian distance:** | ----------*km*---------- | | | | | | | | |
| **Observed Mean:** | 12.59 | 14.54 | 14.62 | 20.84 | 9.94 | 15.79 | 15.79 | 12.42 | 13.78 |
| **Expected Mean:** | 10.43 | 10.29 | 9.54 | 11.21 | 9.53 | 9.85 | 9.85 | 7.80 | 7.68 |
| **Ratio:** | 1.21 | 1.41 | 1.53 | 1.86 | 1.04 | 1.60 | 1.60 | 1.59 | 1.79 |
| **z-score:** | 1.58 | 2.24 | 3.95 | 4.35 | 0.18 | 3.46 | 3.46 | 3.40 | 4.55 |
| **p-value:** | 0.114 | *0.025 | *< 0.001 | *< 0.001 | 0.85 | *0.001 | *0.001 | *0.001 | *< 0.001 |
| **Pattern:** | **Random** | **Dispersed** | **Dispersed** | **Dispersed** | **Random** | **Dispersed** | **Dispersed** | **Dispersed** | **Dispersed** |

* : 0.05 alpha of p-value significance / †Cleth = Clethodim; Pinox = Pinoxaden; Quiz = Quizalofop-p-ethyl; Glyph = Glyphosate; Paraq = Paraquat; Mesos= Mesosulfuron; Fluf = Flufenacet; Metr = Metribuzin; Pron = Pronamide.

# CHAPTER 3: Application of synthetic auxin herbicides to suppress viability of Italian ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) seeds in tall fescue (*Schedonorus arundinaceus (*Schreb*.)* Dumort.) seed production


## ABSTRACT

**BACKGROUND:** Italian ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) is one of the most troublesome weeds worldwide due to the rapid evolution of herbicide resistance. Oregon tall fescue seed production requires high seed purity which demands good control of Italian ryegrass. Thus, the necessity to control herbicide resistant Italian ryegrass and maintain seed purity created interest in new chemical management options. Seven different synthetic auxin herbicide treatments were applied at two growth stages (anthesis and boot) to Italian ryegrass and tall fescue: Two rates of dicamba and 2,4-D, aminopyralid, 2.4-D + clopyralid and, halauxifen-methyl. The objective of this study was to assess the effects of synthetic auxin herbicides on seed viability in biotypes of Italian ryegrass and to assess the feasibility of this management strategy in tall fescue seed production.

**RESULTS:** Only aminopyralid treatments reduced seed quality of Italian ryegrass both in controlled and field environments by reducing the seed viability and seed weight more than 50%. Seed vigor was not affected by the treatments. Speed of germination was affected by the treatments aminopyralid, dicamba and 2,4-D + clopyralid by 2 to 5 days in reduction of germination speed. Aminopyralid treatments had a greater effect when applied at the anthesis stage and had a greater negative impact on tall fescue.

**CONCLUSION:** Aminopyralid applied at anthesis and boot stages to Italian ryegrass

reduced the viability of seeds. Nonetheless, tall fescue plants were more susceptible to aminopyralid, so it is not feasible as a management tool in this crop. Future studies are needed to understand the mechanism of action involved in the seed viability reduction and test the possibility of applying this management practice in other cropping systems.

## Introduction

Weed management has been an important part of agricultural environments since 10,000 B.C [1] and weeds are one of the major causes of yield losses in many crops. Modern agriculture has relied on herbicides to control weeds since the 1940s when the herbicide 2,4-D came to the market.[2] The overuse of herbicides over the last eight decades has created a strong selection pressure for herbicide resistant weed biotypes reducing the efficacy of the herbicide treatments. There are 500 unique cases of herbicide resistance documented worldwide within 256 species (Heap I. (www.weedscience.org). Italian ryegrass (*Lolium perenne* spp. *multiflorum*) is one of the most troublesome resistance weeds due to the high frequency of documented cases of multiple and cross resistance [3] and some physiological characteristics such as cross-pollination that allows the rapid spread of some weedy traits.[4–6]

Once herbicide resistant Italian ryegrass populations become established, the management and the control of those biotypes can be a challenge. If Italian ryegrass survives an herbicide treatment, it can produce between 2,000 to 6,000 seeds per plant causing a large increase to the seed bank intensifying the problem for the next seasons. [7–9] Weed seed banks can be one of the major sources of weed persistence; [10,11] thus, control and management of the seed bank can be a positive way to reduce herbicide resistance

biotypes in a field. There are management techniques that can minimize and reduce the size of a seed bank such as crop rotation and tillage.[8,11,12] Yet, managing late season escapes and preventing seed production could be an option to reduce the weed seed bank.

Previous research in weeds in rangeland areas showed that synthetic auxins can affect seed viability and be used as a management tool to reduce the seed production of invasive annual grasses such as downy brome (*Bromus tectorum*).[13,14] These herbicides kill dicotyledonous plants by generating an auxin overdose which cause an alteration on the cytoskeleton causing epinasty and altering the movement of peroxisomes and mitochondria creating an environment vulnerable to ROS (Reactive Oxygen Species).[15] ROS will be generated due to the increased auxin level that will then activate TIR1/AFB1-5 proteins triggering genes responsible for the production of ethylene and ABA (abscisic acid).[16–18]

The symptomology is not usually observed in mocotyledoneous plants since ABA, ethylene, and production of ROS were shown to be insufficient to lead to death in grasses.[15,19,20] Though, the auxinic herbicide quinclorac controls some grass species such as *Echinocloa, Digitaria* and *Braquiaria* species[20] indicating a possible variation among grass species for response to some auxinic herbicides. Synthetic auxin herbicides sterilize seeds of grasses species like wheat and corn when applied late in the season.[14,21–23] Another indication that the response in grasses may vary within species was shown in previous research that perennial grasses may be more tolerant to late applications of synthetic auxins.[14,24,25]

Hormonal balance and ratio are crucial during seed development. Previous research with *Arabidopsis thaliana* showed that ABA plays a large role on seed development,

dormancy and the formation of endosperm. The ABA present in the seed is synthetized in the maternal plant and zygotic tissue indicating that increasing the ABA production in the maternal plant during seed development can impact seed development.[26,27] Previous research indicated that alterations on the activity of the auxin response factor 2 gene (ARF2) also can affect the seed size on *Arabidopsis thaliana.*[28]

Grass seed growers in Oregon have been raising questions regarding the control of late season escapes of Italian ryegrass in tall fescue (*Festuca arundinacea* Schreb.) fields grown for seeds. Because previous research showed that perennial grasses are less susceptible than annual grasses to synthetic auxin treatments and can vary within species, it is hypothesized that use of a synthetic auxin herbicide late in the season could reduce the seed viability of Italian ryegrass and the seed bank on the next growing season without reducing the viability of tall fescue seeds.

The objectives of this study were to evaluate the effects of synthetic auxin herbicides applied late in the season at different growth stages on seed viability of different biotypes of Italian ryegrass and for tall fescue crop safety.

## Material and Methods

### Site descriptions – field trials

The first experiment was established at the Hyslop Experimental Farm - Oregon State University (44°37'57.1"N; 123°11'38.1"W) between February and July of 2017. Two study sites were used: one area was a recently planted Italian ryegrass and one established second-year tall fescue. The soil is an Amity silt loam with 3.0% of organic matter and pH of 6.2. Average annual precipitation was 1,092 mm with an average annual

temperature of 12°C.

The second experiment was conducted between February and June of 2017 in a three-year tall fescue field infested with Italian ryegrass located north of Dallas, OR (45°02'54.3"N; 123°19'45.6"W). The soil in the location was a Dayton silt loam with 3.3% of organic matter and pH of 5.9. Average annual precipitation ranged from 1,016 to 1,143 mm and an average temperature of 12°C.

The third experiment was conducted at the Schmidt farm - Oregon State University (44°37'38.3"N, 123°12'45.3"W) between March and July of 2018 in a three-year tall fescue field and another area recent planted with Italian ryegrass. The soil in the location is a Woodburn silt loam with 4.0% of organic matter and pH of 5.7. The average annual precipitation is 1,092 mm with an average annual temperature of 12°C.

The fourth experiment was conducted between April and July 2018 in a four years old tall fescue field infested with Italian ryegrass close the flowering period near Gaston, OR (45°27'53.2"N 123°08'43.9"W). The soil in the location is a Helvetia silt loam with 3.5% of organic matter and pH of 6.2. The average annual precipitation is 1,143 mm with an average annual temperature of 14°C.

## Plant material and establishment

### *Greenhouse experiments*

The experiments were conducted in 2017 and 2018 in greenhouses located at Corvallis, OR (44°34'03.7"N 123°17'10.3"W). Four greenhouses were used to separate Italian ryegrass populations to produce seeds for viability comparison. The greenhouse was set at a photoperiod of 16 h photoperiod and a temperature range between 18 and

21ºC.

Four biotypes of Italian ryegrass were used. The biotypes came from different areas in the Willamette Valley and had different types of herbicide-resistant: glyphosate-resistant (‘PR’) (44°57'21.1"N 122°53'01.6"W), ACCase-resistant (‘RD’) (45°28'58.9"N 123°03'20.4"W), ALS-resistant (‘TF’) (44°43'37.4"N 122°50'29.9"W) and a susceptible biotype (‘FG’) (45°33'12.9"N 123°06'55.5"W). The resistant populations were characterized by two replicated dose-response experiments (Appendix B). Seeds from each biotype for this study were produced on greenhouse after characterization.

Seeds of each population were germinated in four germination boxes with size of 11x11x2.8 cm (156C container, Hoffman Manufacturing Inc; Corvallis, OR 97330 USA) in a growth chamber with a photoperiod of 16 hours of light and temperature regime of 21/10°C in May of 2017 for the first experiment and January of 2018 for the second. Seedlings were transplanted into 15.24 by 12.70 cm pots 10 days after germination and kept in the greenhouse. Plants were watered daily, and a 20-20-20 fertilizer was added once a week. The experiment was conducted from January to April of 2017 and repeated in the same months in the year of 2018.

***Field Experiments***

Two areas of 43 by 20 m located in the Hyslop Experimental Farm were sown with a turf type tall fescue (Rebel XLR cultivar; NJAES/Rutgers University) and the other with a commercial Italian ryegrass cultivar (Florida 80; NJAES/Rutgers University). The Italian ryegrass was planted using a disc-drill on April 9, 2017, at 18 kg/ha at a depth of 0.64 cm and 35 cm row spacing. The tall fescue was planted using a disc-drill at 20 kg/ha on April 8, 2015, at a depth of 0.64 cm and row spacing of 45 cm. Two applications of

40-0-0 fertilizer at 90 kg/ha was made in January and March of 2017 on both fields. Fungicide (Quilt Excel Syngenta Crop Protection, Greensboro, NC) was sprayed to control rust. Weeds were pulled by hand as needed. Irrigation was used as needed. Individual plots size was 3.048 by 10.363 m.

Another study was conducted in a second-year turf-type tall fescue field near Dallas, OR.The cultivar was a AST 5112 (Allied Seed, LLC; 9311 Highway 45 Nampa, ID 83686 United States) planted with a row spacing of 25 cm. The field had a high infestation with Italian ryegrass which wore kept to test the effects of treatment in a natural population. The area was fertilized with Urea at 20 kg/ha and treated for slugs in October 2016. In February 2017, the area received another fertilization treatment of 30 kg/ha of 33-0-0-12 and 68 liters of liquid nitrogen. In May 2017, the area was treated with fungicide (Quilt Excel Syngenta Crop Protection, Greensboro, NC) and plant growth regulator (Palisade EC, Syngenta Crop Protection, Greensboro, NC) according to label. Plots size was 3.048 by 10.363 m.

At the Schmidt Farm experiment two adjacent areas of 10 by 32 m were carbon seeded with a turf type tall fescue (Rebel XLR cultivar; NJAES/Rutgers University) in September 2015 at a depth of 0.64 cm and a row space of 35 cm using a disc drill calibrated to deliver 18 kg/ha of seeds. Two broadcast application of nitrogen were made in January and March 2018 at 100 kg/ha and 80 kg/ha of N 40-0-0, respectively. Another adjacent area of 10 by 42 m was planted with Italian ryegrass commercial cultivar (Florida 80; NJAES/Rutgers University) using a disc-drill on April 27 2018, calibrate to deliver 20 kg/ha at a depth of 0.65 cm and 35 cm of row spacing. One application of 40-0-0 fertilizer at 90 kg/ha was made in May of 2018. Weeds were pulled by hand as

needed. The area was treated with fungicide (Quilt Excel Syngenta Crop Protection, Greensboro, NC) and plant growth regulator (Palisade EC, Syngenta Crop Protection, Greensboro, NC) according to label. Irrigation was used as needed. Individual plots were 1.83 by 3.65 m.

Two adjacent areas of tall fescue with 10 by 32 m near Gaston, OR, were used. The cultivar was Penn RK4 (Pennington Seed Inc.; 270 Hansard Ave, Lebanon, OR 97355) planted in 2010. For the year that the trial was conducted, two applications of nitrogen were made: 44.83 kg $ha^{-1}$ in the fall of 2017 and 145.71 kg $ha^{-1}$ in the spring of 2018.The area was treated with fungicide (Quilt Excel Syngenta Crop Protection, Greensboro, NC) and plant growth regulator (Palisade EC, Syngenta Crop Protection, Greensboro, NC) according to label.

## Treatments

Eight synthetic auxin herbicides (WSSA - Group 4) were sprayed in two different growth stages (BBCH 49 boot stage and BBCH 59 anthesis stage) of both the crop and weed to test the effects of each treatment on different late growth stages of the plants (Table 3-1).

During the 2017 trials, the higher rates of dicamba, 2,4-D, and the florasulam plus halauxifen-methyl treatments were not sprayed. During the Dallas 2017 trial, the aminopyralid treatment was not applied. All treatments were applied with a 90% non-ionic surfactant (NIS) at a rate of 0.25% v/v as recommended by the labels. For the greenhouse trials, each plant was separately sprayed using an air cabinet sprayer (Generation III Spray Chamber, De Vries Manufacturing, 86956 State HWY 251,

Hollandale, MN 56054) with a flat-fan spray *Teejet nozzle 8004* set to deliver 187 L $ha^{-1}$. Nozzles were set at 63.5 cm from the target. For the field trials, a $CO_2$ backpack sprayer with 0.9144 m boom with 3 *Teejet nozzles 8004* with a 45.72 cm spacing calibrated to deliver 187 L $ha^{-1}$. After the application, seeds were allowed to mature and harvested when they reached a seed moisture around 35%. A quadrat was used to harvest 1 $m^2$ of each plot at each field trial. Seeds from each pot were harvested in the greenhouse trial. Seeds were cleaned for testing.

## Experimental design

All greenhouse, Gaston and Dallas trials were made using a complete randomized block design (Figure B.7 and Figure B.9) with four blocks (field trial) and six blocks (greenhouse) for each treatment (herbicide + growth stage). For the Gaston and Dallas field trials, each plot was considered a block, and for the greenhouse trials, six pots (15.24 by 12.70 cm) were considered a block. Treatments consisted of growth stage plus herbicide treatment.

The Hyslop and Schmidt trials were strip split randomized block designs (Figure B.8) with herbicides as primary treatment, and growth stage as secondary treatment. Plots on the field trials were established so that no soil variation, shade or other abiotic factors could generate differences among blocks. Randomization was generated using the ARM (Agriculture Research Manager) software (Gylling Data Management, Inc.; 405 Martin Boulevard Brookings, South Dakota 57006-4605 USA).

## Seed and pollen viability tests

After harvest, seeds of each plot in both greenhouse and field trials were used for seed

testing. The purpose of these trials was to determine if the late herbicide treatments affected the viability and vigor of the seeds. Five experiments were done: a seed germination test plus a tetrazolium test, a speed of germination test, an accelerated aging test, a pollen germination test, and a seed weight variation test. Seed germination, tetrazolium and accelerated aging test were conducted according to the Association of Official Seed Analysts (AOSA).[30,31]

***Germination, tetrazolium and speed germination test***

Seed viability was evaluated using a standard seed germination test with four replications. In this trial, 100 seeds were placed in standard Petri dishes containing blue paper blotters soaked in distilled water. The Petri dishes were place in sealed plastic bags to avoid loss of water and placed in a germination chamber set with a photoperiod of 16 hours with a light intensity of 700 flux and day temperature regime of 21ºC and 15ºC during light and dark periods, respectively. Seeds were kept in the chamber for 14 days and five germination counts were made (3, 5, 7, 10 and 14 days); seeds that germinated were removed on each count. Seeds were considered germinated if both radicle and coleoptile were visible. After the last count, seeds that did not germinate were tested using a tetrazolium to determine the seed viability. Viability was considered the sum of seeds that germinated on each count plus the seeds that were viable in the tetrazolium test.

***Accelerated aging test***

According to INSTA (International Seed Testing Association), seed vigor is commonly defined as the ability of a seed to be able to perform well under environmental

conditions that are not optimal for the studied species.[32] To determine the effects of the herbicide treatments on the seed vigor, an accelerated aging test was conducted to induce stress into seeds with a high temperature and high relative humidity. Twenty-five ml of distilled water were placed inside each four germination boxes with size of 11x11x2.8 cm (156C container, Hoffman Manufacturing Inc; Corvallis, OR 97330 USA), and accelerated aging trays (Hoffman Manufacturing Inc; Corvallis, OR 97330 USA) were placed inside the boxes and 1 g of seeds added. Germination boxes were placed inside a growth chamber in the dark and set to a temperature of 41ºC; Italian ryegrass seeds were kept in the chamber for 48 hours and the tall fescue seeds for 72 hours. Four replications of 25 seeds each were taken from the aged seeds, and a germination test was conducted as described previously. Results were compared with the previous germination trial to determine if there were any differences.

***Seed weight test***

Seed weight of 1,000 seeds was measured with two subsamples for each replication (Greenhouse and field). A seed counter (Old mill seed counter Model 850-2; International Marketing and Design Corp. 13802 Lookout Road, Suite 200, San Antonio, TX 782330) was used to count the 1,000 seeds samples.

***Pollen viability***

This assay was only conducted for the greenhouse trials. To test the effects of the herbicide treatments on pollen viability, pollen was collected from each block two weeks after treatment. A 47mm petri-dish containing an artificial media [33] was placed in the middle of each block and plants were shaken to collect the pollen to the media. After 30

min in contact with the media, the petri-dishes were put under a microscope. One hundred randomly choose pollen grain were counted. Pollen tube with a diameter longer than the pollen grain was considered to have germinated [33–35].

## Data analysis

Field trials were analyzed separately due to the differences between experimental design and treatment numbers. Greenhouse trials were first analyzed separately according to year and then if the assumption of homogeneity was met, pulled together for the treatments that were the same in both years. Before fitting the models, data structure was analyzed for assumption checking of normality and homogeneity using diagnostic plots and Levene's test. Diagnostic plots showed that data were over dispersed ($Var(Y) > \mu y$) with some normality issues. Data was modeled using a generalized linear mixed model via PQL (Penalized Quasi-Likelihood) to account for data over dispersion [35] using as fixed effects herbicide treatments, growth stage and species/biotypes and their interactions to explain the response variables. Blocking factor and possible variability on growth stage and species/biotypes were counted as random effects in the model. The response variables were seed viability, seed vigor reduction, and seed weight. For the greenhouse trial, the same response variables were used with the addition of pollen viability. The data were subjected to an analysis of deviance using a Wald Chi-square test type test II procedure and mean differences were quantified using an HSD Tukey's test at a 5% significance level.

Inferences about the speed of germination were made using the germination data collected from each evaluation day on the standard germination trial and fitting the data

on a three-parameter log-logistic regression [2] [36]

$$y = c + \frac{GermMax - c}{1 + \left(\frac{x}{TD_{50}}\right)^{b}} \quad [2]$$

where *y* is the response, *GermMax* refers to the maximum germination and *c* to the lower limit of the sigmoid curve, $TD_{50}$ denotes the time in days, x refers to the time to 50% of the seeds germinate between *GermMax* and *c,* and *b* refers to the relative slope of the curve around $TD_{50}$. The ratio of $TD_{50}$ of each treatment with the control was used to assess the effects over germination speed. Analyses were made using the R software (R Foundation for Statistical Computing (https://www.R-project.org/) with the packages, lme4 (Bates D, Mächler M, Bolker B, Walker S. (http://arxiv.org/abs/1406.5823), Package MASS[37], Multcomp[38], Tidyverse packages (Hadley H. (https://CRAN.R-project.org/package=tidyverse) and drc package.[39]

## Results

### Greenhouse experiments

The effects of synthetic auxin herbicides on the different Italian ryegrass biotypes indica similar results for both years. In the 2017 trial, analysis of deviance showed that seed viability response to the treatments was significant ($p$-value < 0.05) with a significant interaction of treatment and biotypes variables (Table B.4). Further analysis of mean comparison between treatments showed that only aminopyralid reduced seed viability but with differences among populations (Figure 3.1). Overall, the viability reduction caused by aminopyralid was between 29 to 48% when applied at anthesis and 32 to 54% when applied at the boot stage. FG biotype was the most susceptible to

aminopyralid treatments at both growth stages. Seed weight variation followed a similar pattern to viability but with only differences of treatment and populations (Table B.6); seed weight was only reduced by aminopyralid treatments. A reduction in seed weight (Figure 3.3) of almost 50% was observed in all populations with no differences between the growth stages. Seed vigor on the other hand, was not uniform due to a large variability between biotypes (Table B.8).

TF biotype was the most affected by the stress test where all treatments, including the control, had large reductions in viability after stress exposure. Yet, seeds from plants treated with 2,4-D and dicamba resulted a larger viability reduction in the TF population. Speed of germination was not uniform among the treatments and biotypes (Figure 3.9, Figure 3.11); Results indicate that synthetic auxins could have a small effect on speed of germination but with no significance in terms of weed management.

For the 2018 trial, similar results to the 2017 trial were observed; the three added treatments were not different compared to the control and the other treatments except aminopyralid. When aminopyralid was applied in the anthesis stage, it reduced the seed viability by 80% (Figure 3.2) in most biotypes; only the TF biotype showed less reduction of around 60%. Plants sprayed with aminopyralid at the boot stage in 2018 were less affected by the treatment than in 2017. Seed weight was reduced by the aminopyralid treatments (Figure 3.4) with an overall reduction of around 50% with no differences among the populations or stages (Table B.7). Seed vigor results were similar to the ones observed in 2017 (Figure 3.6) with only differences are among populations. There was no differences in speed of germination among treatments, biotypes and stages (Figure 3.12, Figure 3.14). Maximum germination analysis agreed with results observed

on viability analysis in both years showing a reduction in the maximum germination in aminopyralid treatments (Figure 3.10, Figure 3.12). For pollen viability, no effect was observed among treatments and biotypes in either year. Pollen viability ranged between 94% to 99% (Table B.10, Table B.11)

Because the added treatments in 2018 were not different from the control, these treatments where dropped from the analysis and data of the treatments used in both years were combined. Data of both years confirm the results of aminopyralid treatment. Seed viability analysis of deviance (Table B.12) showed an interaction between treatment, biotype and stage. Aminopyralid reduced seed viability between 60 to 70% for the anthesis treatment; however, seed viability from plants treated at the boot stage was less affected by aminopyralid (Figure 3.7). Seed weight data was not combined due to the homogeneity assumption was not met among year results. Seed vigor effect was only significant at the biotype level (Table B.13) where only the TF biotype had a lower seed vigor but with no relation to treatments (Figure 3.8).

## Field experiments

Despite the differences in experimental design and number of herbicide treatments, the results were similar in all field trials. Aminopyralid treatments were the only ones that reduced seed health.

In the Dallas OR, 2017 field trial no differences were observed in both seed viability and seed weight (Table B.14, Table B.18, Figure 3.15 and Figure 3.19). For both species treated with 2,4-D and 2,4-D + clopyralid there was a decrease in seed viability after the seed vigor test; however, not different to the control. Regarding speed of germination,

round 25 to 30% increase on germination time for Italian ryegrass at the anthesis stage treated with 2,4-D and 2,4-D + clopyralid but this only represents an increase of two days which, from a management point of view, would be not be great enough to make this treatment viable. No differences were observed for tall fescue (Figure 3.27, Figure 3.29). Maximum germination results indicate no differences and without losses in viability (Figure 3.15, Figure 3.28).

For the Hyslop Farm 2017 trial in Corvallis, OR, in contrast to Dallas, OR, trial there were differences among treatments with significant differences between species (Table B.15 and Table B.19). The seed viability test showed a larger susceptibility of tall fescue to aminopyralid (Figure 3.16) with a reduction between 80% at the boot stage and 90% at the anthesis stage while Italian ryegrass had a reduction of 60% at the boot stage and 70% at the anthesis. The same pattern was observed for seed weight (Figure 3.20). A larger effect of aminopyralid was observed in both species during the boot stage with a reduction of around 40% on seed weight and, at the anthesis stage, reduction of around 20% was documented.

Losses of seed vigor after the aging test were different between species when treated with aminopyralid at the Hyslop trial (Table B.23). Results showed a reduction of 10% on Italian ryegrass treated at boot stage and 4% at the treated at anthesis stage; there were no effects on tall fescue seed vigor (Figure 3.24). For germination speed, an increase of 77% (4 days) was observed on both stages of Italian ryegrass treated with aminopyralid (Figure 3.30 and Figure 3.32); however, this could not be large enough increase in germination time for management purpose (Table B.51). Maximum germination followed the same patterns observed on the viability tests, a decrease in germination was observed

in the seeds from plants treated with aminopyralid with a greater effect on tall fescue plants (Figure 3.31).

The Gaston, OR, 2018 trial had three treatments added compared to the 2017 trials. Analysis of deviance showed a large variation with interaction between all factors for the seed viability test (Table B.16, Table B.20 and Table B.24). Seed viability was affected by aminopyralid and with differences among species sprayed at anthesis. Italian ryegrass had a reduction of 50% in seed viability while tall fescue had a reduction of 70%; both species had a reduction of 40% when aminopyralid was sprayed at the boot stage (Figure 3.17). No differences were observed for aminopyralid treatments between species and stages for seed weight with an average reduction of 34% when compared to the control (Figure 3.21). Viability reduction was variable among the treatments in both species with no differences between the treatments and the control (Figure 3.25). Speed of germination test results from this trial had some differences from other field trials with a larger variability among treatments (Figure 3.33, Figure 3.35). Dicamba treatments showed an increase of 70% in germination time and 50% for 2,4-D + clopyralid treatment in Italian ryegrass when treatments were applied at the anthesis stage. On the Italian ryegrass sprayed with aminopyralid at the boot stage showed a reduction in speed of germination. However, due to lack of similarities with the results of other field trials, these differences are more likely due to difference in the plant biology of the Italian ryegrass population on this specific location of the trial than to effects of the herbicide treatments. Maximum germination results showed a reduction from aminopyralid treatments similar to the ones observed in the viability analysis (Figure 3.34).

Similar to the trial in Gaston, the Schmidt Farm experiment had three additional

treatments in comparison to the 2017 trials. Analysis of deviance showed significance interaction between the three analyzed factors (Table B.17, Table B.21 and Table B.25). Mean comparison results (Figure 3.18) showed that aminopyralid, like in the other field trials, affected the viability of the seeds in both species (Figure 3.18). However, tall fescue plants treated with aminopyralid at the anthesis stage had a reduction of 72% viability compared to the effects on Italian ryegrass that showed a reduction of 42%. No difference was observed between growth stages on the effect on seed viability of aminopyralid in Italian ryegrass. Both species were affected equally when aminopyralid was applied at the boot stage. Seed weight reduction was only affected by aminopyralid with no differences between species or growth stage; the average seed weight reduction was between 50 to 60% (Figure 3.22). Seed vigor reduction after the stress test showed a large effect of the aminopyralid when sprayed at the anthesis stage on Italian ryegrass (Figure 3.26).

For speed of germination, no differences were observed among treatments, species and growth stages; maximum germination tests had similar results to viability test results (Figure 3.36, Figure 3.38).

## Discussion

The greenhouse study results showed that the aminopyralid can be used to reduce the viability and weight of seeds of different Italian ryegrass biotypes. Some of the biotypes had poor germination prior to treatments making the viability reduction effects less evident. These results are in contrast with previous research that showed the effects on seed viability other grass species such as downy brome [14,40] where it was documented that

benzoate and phenoxy-carboxylates herbicides reduced viability of this species. The same results were not observed in Italian ryegrass in multiple biotypes strengthening the hypothesis that the effect of synthetic auxin herbicides vary within grass species and among herbicide chemical families in this herbicide group.

Results observed in the field studies were similar to the results observed in the greenhouse. Aminopyralid affected the seed viability of Italian ryegrass; however, the larger effect on the tall fescue makes this strategy non feasible to control Italian ryegrass. These results show a contrast with information of previous research that indicates that perennial grasses are generally less susceptible to synthetic auxin treatments than annual grasses [14,24,25]. These results indicate differences of susceptibility within grass species.

Results showed that some synthetic auxin herbicides, such as aminopyralid, 2,4-D and 2,4-D + clopyralid, can affect the speed of germination; however, the results only point out differences of 3 to 5 days of delay indicating that this effect is not enough to be considered from a weed management point of view.

Aminopyralid is currently registered for rangelands, pastures and some non-crop areas and was previously shown to reduce the viability of other grass species such as downy brome and medusahead.[14,40,41] Despite the fact that this herbicide is not registered for some cropping areas, our results show that this herbicide could successfully reduce the viability of Italian ryegrass seeds and minimize the issues with the weed in the next season. Large variability observed in the results may indicate that an optimum rate for this herbicide still needs to be selected with further tests. Previous research showed that grasses [42] such as tall fescue do not translocate some herbicides from the aminopyralid chemical family indicating that spray coverage can be an important factor to manage seed

viability of Italian ryegrass seeds.

In some crops, such as orchards and tree crops, the use of synthetic auxin herbicides should be carefully planned due to the risk of off-target movement. Despite the drift potential, aminopyralid has been shown to be one the less volatile herbicides in its group.[43,44] This indicates a potential for aminopyralid to be considered for further studies of crop safety in areas where the herbicide will not have a direct contact with the crop such as in perennial orchard crops. These types of crops have had herbicide resistant Italian ryegrass for years [3,5] and the possibility of reducing the viability of seeds of late season escapes could be an option to manage the presence of resistance.

Previous studies also show that aminopyralid could be used as a pre-emergent herbicide to control grasses [41] and have a low sorption on the soil [45]. Future studies could look at the effects over Italian ryegrass seeds, and its applicability. This current project did not study the effects on the seed bank in the following years after late treatments with aminopyralid; however, the current results could serve as background for future studies to evaluate the effects on Italian ryegrass seed banks.

Aminopyralid was the only one of the tested herbicides in this study to show an effect in seed viability. These results raise questions about how different synthetic auxin products affect seed development. Some previous works have shown how natural auxin (IAA) can regulate seed development and seed size [26,28] by regulating and starting ABA biosynthesis. However, there are no studies showing the difference and comparison between synthetic auxin molecules on the effect of seed development. A previous study with *A*. *thaliana* showed that it was possible to have a mutation in an auxin receptor homologs AFB5 and in SGT1b which confer resistance to picolinate auxins but not to

2,4-D; and how the numerous relationships between these receptors, auxin and many physiological processes present in plants such as seed and embryo development. [46,17] Thus, this could be an indication that different synthetic auxin molecules could have different auxin receptors involved in the mechanism affecting seed viability.

## Conclusions

Based on results from field and greenhouse trials, aminopyralid reduced seed viability, seed weight and, in some cases, seed vigor of Italian ryegrass. Some differences were observed among Italian ryegrass biotypes indicating a possible larger tolerance to this treatment in some biotypes. These results indicate that an optimum rate still needs to be found to affect a larger number of biotypes. In contrast to previous research, this study did not document that benzoate and phenoxy-carboxylates herbicides reduced viability of Italian ryegrass seed. Further studies are needed to understand the different effects of different chemical molecules of synthetic auxin herbicides on seed development. Field studies also showed that aminopyralid was the only effective treatment to reduce seed health. However, the effects on tall fescue were greater than on the target weed, making this management strategy unfeasible in tall fescue seed production. This result also contrasted with previous research that indicated that perennial grasses generally suffer less damage than annual grasses to synthetic auxin herbicide treatments. This study will serve as basis for future studies to understand the effects and crop safety of this management strategy on crops that do not have direct contact with the treatment such as perennial orchards and to also understand the effects on seed banks.

# References

1. Hay J. Gains to the grower from weed science. *Weed Science*. **22**:439–42 (1974).

2. Busi R, Goggin DE, Heap IM, Horak MJ, Jugulam M, Masters RA, et al. Weed resistance to synthetic auxin herbicides. *Pest Management Science*. **74**:2265–76 (2018).

3. Brunharo CA, Hanson BD. Multiple herbicide–resistant Italian ryegrass [*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot] in California perennial crops: characterization, mechanism of resistance, and chemical management. *Weed Science*. **66**:696–701 (2018).

4. Giddings G. Modelling the spread of pollen from *Lolium perenne*. The implications for the release of wind-pollinated transgenics. *Theoretical and Applied Genetics*. **100**:971–4 (2000).

5. Karn E, Jasieniuk M. Genetic diversity and structure of *Lolium perenne* ssp. *multiflorum* in California vineyards and orchards indicate potential for spread of herbicide resistance via gene flow. *Evolutionary Applications*. **10**:616–29 (2017).

6. Meyers SC. Flora of Oregon. Volume 1, Pteridophytes, gymnosperms, and monocots. 1st ed. Vol. 1. Fort Worth, Texas: Botanical Research institute; pp. 591 (2015).

7. Steadman KJ, Ellery AJ, Chapman R, Moore A, Turner NC. Maturation temperature and rainfall influence seed dormancy characteristics of annual ryegrass (*Lolium rigidum*). *Australian Journal of Agricultural Research*. **55**:1047–57 (2004).

8. Bagavathiannan MV, Norsworthy JK. Late-season seed production in arable weed communities: management implications. *Weed science*. **60**:325–34 (2012).

9. Young FL, Whitesides RE. Efficacy of postharvest herbicides on Russian thistle (*Salsola iberica*) control and seed germination. *Weed Science*. **35**:554–9 (1987).

10. Cousens R, Mortimer M. Dynamics of weed populations. Cambridge University Press; pp. 332 (1995).

11. Davis AS. When does it make sense to target the weed seed bank? *Weed Science*. **54**:558–65 (2006).

12. Buhler DD, Hartzler RG, Forcella F. Implications of weed seedbank dynamics to weed management. *Weed Science*. **45**:329–36 (1997).

13. Rinella MJ, Haferkamp MR, Masters RA, Muscha JM, Bellows SE, Vermeire LT. Growth regulator herbicides prevent invasive annual grass seed production. *Invasive Plant Science and Management*. **3**:12–6 (2010).

14. Rinella MJ, Masters RA, Bellows SE. Effects of growth regulator herbicide on downy brome (*Bromus tectorum*) seed production. *Invasive Plant Science and Management*. **6**:60–4 (2013).

15. Christoffoleti PJ, Figueiredo MRA de, Peres LEP, Nissen S, Gaines T, Christoffoleti PJ, et al. Auxinic herbicides, mechanisms of action, and weed resistance: A look into recent plant science advances. *Scientia Agricola*. **72**:356–62 (2015).

16. Badescu GO, Napier RM. Receptors for auxin: will it all end in TIRs? *Trends in Plant Science*. **11**:217–23 (2006).

17. Salehin M, Bagchi R, Estelle M. SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *The Plant Cell*. **27**:9–19 (2015).

18. Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, Estelle M, et al. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature*. **446**:640–5 (2007).

19. Grossmann K. Mediation of herbicide effects by hormone interactions. *Journal of Plant Growth Regulation*. **22**:109–22 (2003).

20. Grossmann K. Auxin herbicides: current status of mechanism and mode of action. *Pest Management Science*. **66**:113–20 (2010).

21. Friesen H, Born WV, Keys C, Dryden R, Molberg E, Siemens B. Effect of time of application and dosage of dicamba on the tolerance of wheat, oats and barley. *Canadian Journal of Plant Science*. **48**:213–5 (1968).

22. Rinella MJ, Kells JJ, Ward RW. Response of 'Wakefield'winter wheat (Triticum aestivum) to dicamba. *Weed Technology*. **15**:523–9 (2001).

23. Sikkema PH, Brown L, Shropshire C, Soltani N. Responses of three types of winter wheat (*Triticum aestivum* L.) to spring-applied post-emergence herbicides. *Crop Protection*. **26**:715–20 (2007).

24. Sheley RL, Duncan CA, Halstvedt MB, Jacobs JS. Spotted knapweed and grass response to herbicide treatments. *Journal of Range Management*. **53**:176–82 (2000).

25. Shinn SL, Thill DC. Tolerance of several perennial grasses to imazapic. *Weed Technology*. **18**:60–5 (2004).

26. Kanno Y, Jikumaru Y, Hanada A, Nambara E, Abrams SR, Kamiya Y, et al. Comprehensive hormone profiling in developing *Arabidopsis* seeds: examination of the site of ABA biosynthesis, ABA transport and hormone interactions. *Plant Cell Physiol*. **51**:1988–2001 (2010).

27. Rock CD, Quatrano RS. The role of hormones during deed development. In: Davies PJ, editor. *Plant Hormones: Physiology, Biochemistry and Molecular Biology [Internet]*.

Dordrecht, Netherlands: Springer; p.671–97 (1995). Available from: https://doi.org/10.1007/978-94-011-0473-9_31

28. Schruff MC, Spielman M, Tiwari S, Adams S, Fenby N, Scott RJ. The AUXIN RESPONSE FACTOR 2 gene of *Arabidopsis* links auxin signalling, cell division, and the size of seeds and other organs. *Development*. **133**:251–61 (2006).

29. Baalbaki R, Elias SG, Marcos Filho J, McDonald, MB. Seed vigor testing handbook. 32nd ed. Ithaca, New York: Association of official seed analysis; pp. 88 (2009).

30. Elias SG, Copeland LO, McDonald MB, Baalbaki RZ. Seed testing: principles and practices. 1st ed. East Lansing, Michigan: Michigan State University Press; pp. 364 (2012).

31. Marcos Filho J, Marcos Filho J. Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola*. **72**:363–74 (2015).

32. Fei S, Nelson E. Estimation of Pollen Viability, Shedding Pattern, and Longevity of Creeping Bentgrass on Artificial Media. *Crop Science*. **43**:2177–81 (2003).

33. Ahloowalia B. Germination in vitro of ryegrass pollen grains. *Euphytica*. **22**:575–81 (1973).

34. Tuinstra M, Wedel J. Estimation of pollen viability in grain sorghum. *Crop Science*. **40**:968–70 (2000).

35. Bolker B. Dealing with quasi-models in R. *Compare*. **1**:5–452305 (2017).

36. Ritz C, Pipper CB, Streibig JC. Analysis of germination data from agricultural experiments. *European Journal of Agronomy*. **45**:1–6 (2013).

37. Venables W, Ripley B. Modern applied statistics with S. 4th ed. New York: Springer; (2002).

38. Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S, et al. Simultaneous inference in general parametric models. *Biometrical Journal*. **50**:346–63 (2016).

39. Knezevic SZ, Streibig JC, Ritz C. Utilizing R software package for dose-response studies: the concept and data analysis. *Weed Technology*. **21**:840–8 (2007).

40. Ball DA. Effects of aminocyclopyrachlor herbicide on downy brome (*Bromus tectorum*) seed production under field conditions. *Invasive Plant Science and Management*. **7**:561–4 (2014).

41. Kyser GB, Peterson VF, Davy JS, DiTomaso JM. Preemergent control of medusahead on California annual rangelands with aminopyralid. *Rangeland Ecology & Management*. **65**:418–25 (2012).

42. Lewis DF, Roten RL, Everman WJ, Gannon TW, Richardson RJ, Yelverton FH. Absorption, translocation, and metabolism of aminocyclopyrachlor in tall fescue (*Lolium arundinaceum*). *Weed science*. **61**:348–52 (2013).

43. Strachan SD, Ferry NM, Cooper TL. Vapor movement of aminocyclopyrachlor, aminopyralid, and dicamba in the field. *Weed technology*. **27**:143–155 (2013).

44. Strachan SD, Casini MS, Heldreth KM, Scocas JA, Nissen SJ, Bukun B, et al. Vapor movement of synthetic auxin herbicides: aminocyclopyrachlor, aminocyclopyrachlor-methyl ester, dicamba, and aminopyralid. *Weed science*. **58**:103–108 (2010).

45. Fast BJ, Ferrell JA, MacDonald GE, Krutz LJ, Kline WN. Picloram and aminopyralid sorption to soil and clay minerals. *Weed science*. **58**:484–9 (2010).

46. Walsh TA, Neal R, Merlo AO, Honma M, Hicks GR, Wolff K, et al. Mutations in an Auxin Receptor Homolog AFB5 and in SGT1b Confer Resistance to Synthetic Picolinate Auxins and Not to 2,4-Dichlorophenoxyacetic Acid or Indole-3-Acetic Acid in *Arabidopsis*. *Plant Physiology*. **142**:542–52 (2006).

# Figures

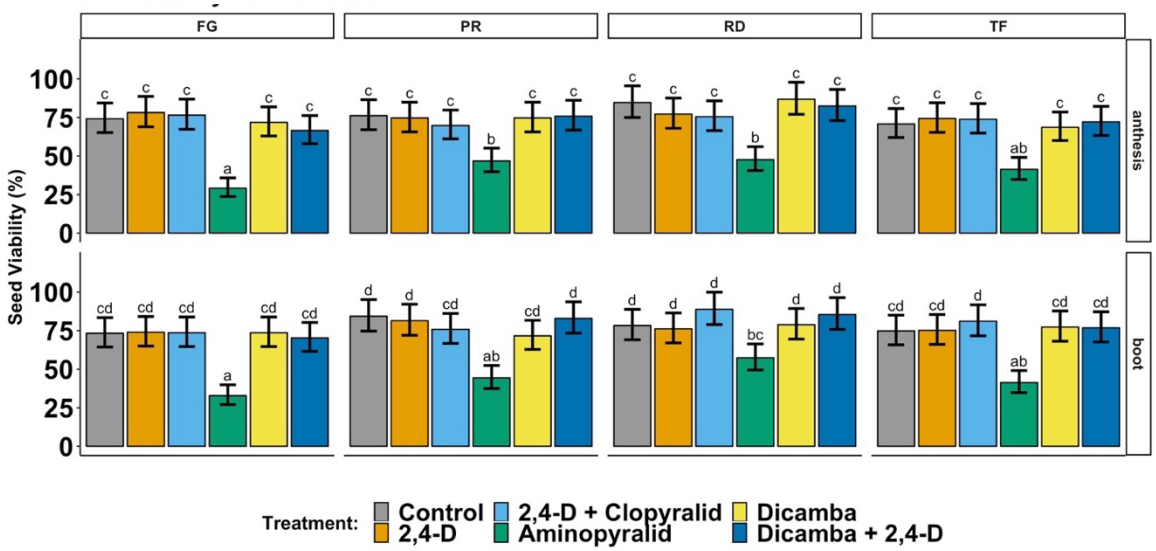



Figure 3-1. Seed viability in response of herbicide treatments at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse trial in 2017. (Letters represent different mean groups on HSD Tukey test p-value < 0.05) Means in Table B.26

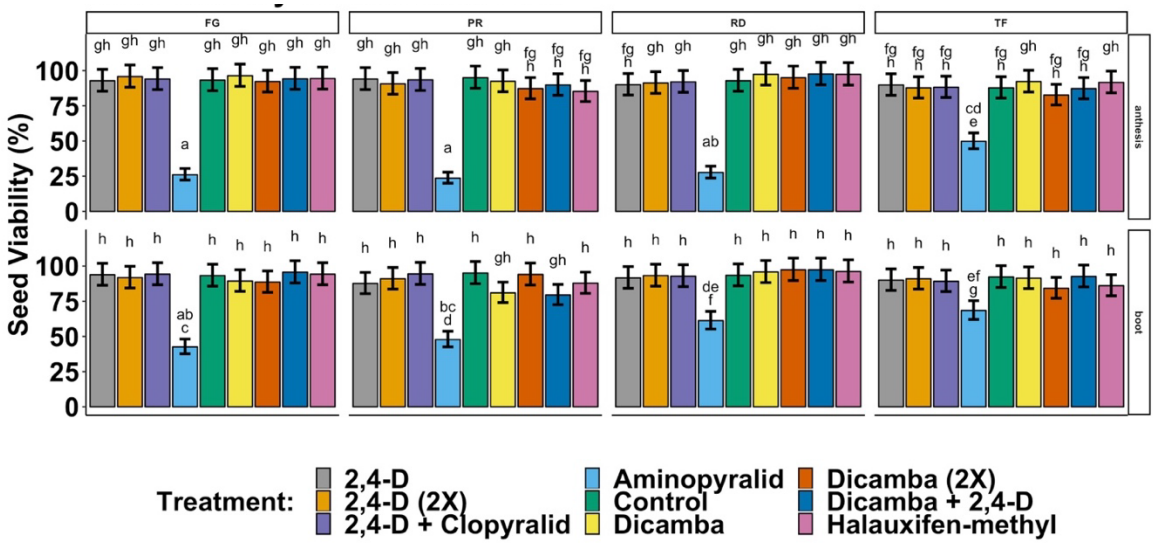



Figure 3-2. Seed viability in response of herbicide treatments at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse trial in 2018. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.27

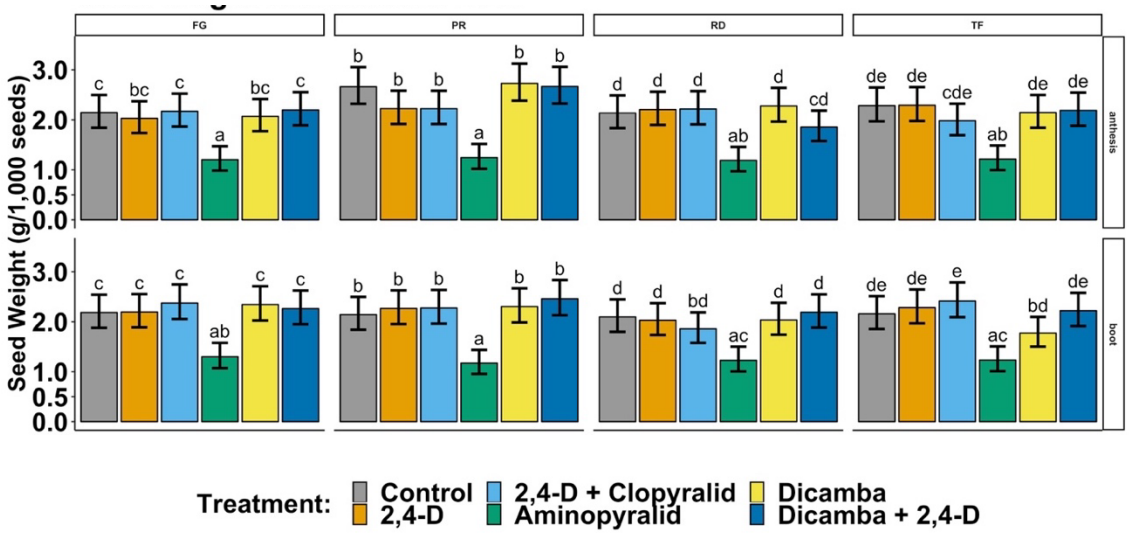



Figure 3-3. Seed weight in response of herbicide treatments at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse trial in 2017. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.28

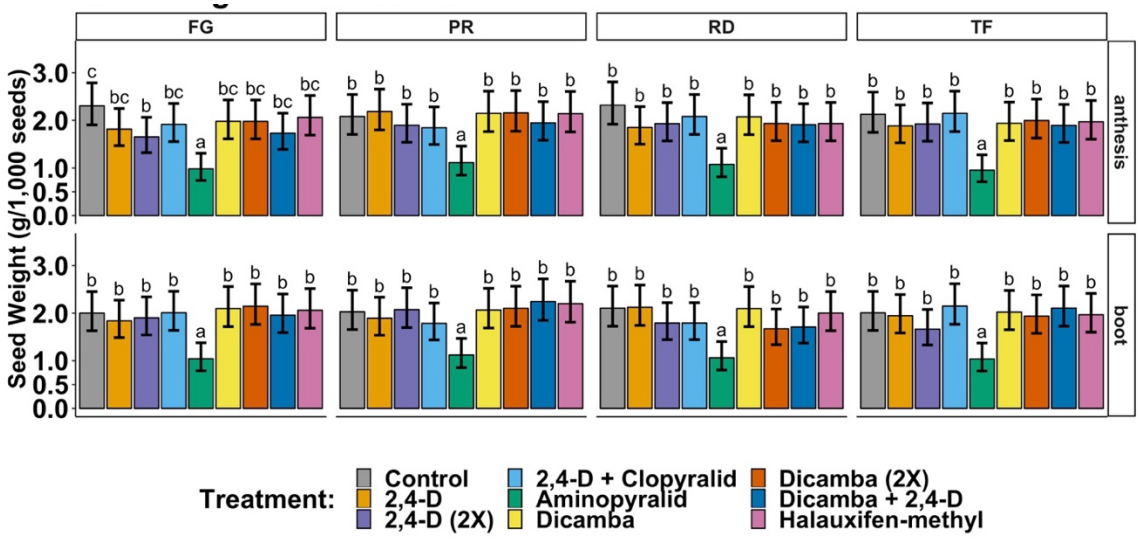



Figure 3-4. Seed weight in response of herbicide treatments at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse trial in 2018. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.29

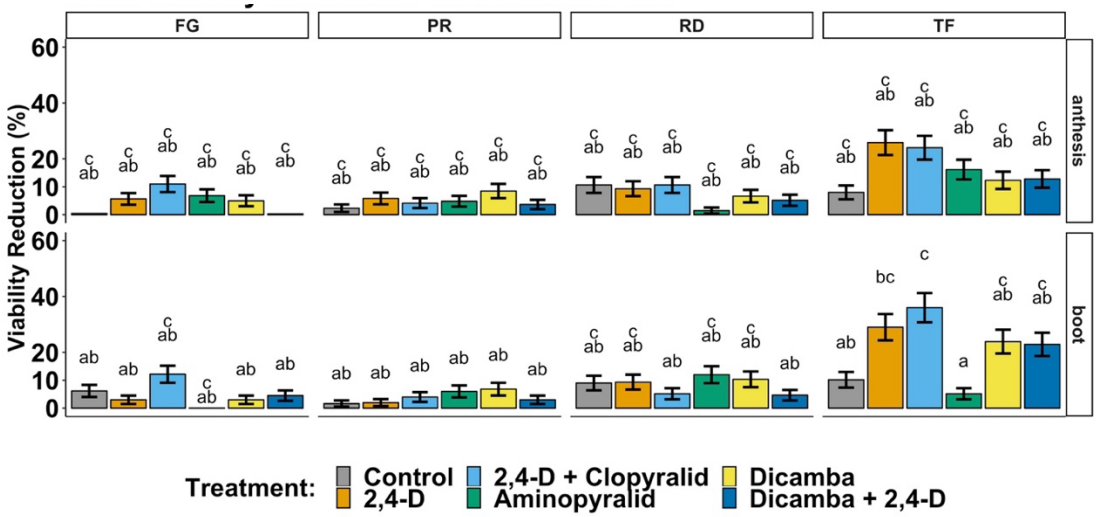



Figure 3-5. Seed viability reduction after accelerated aging test in response of herbicide treatments at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse trial in 2017. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.30

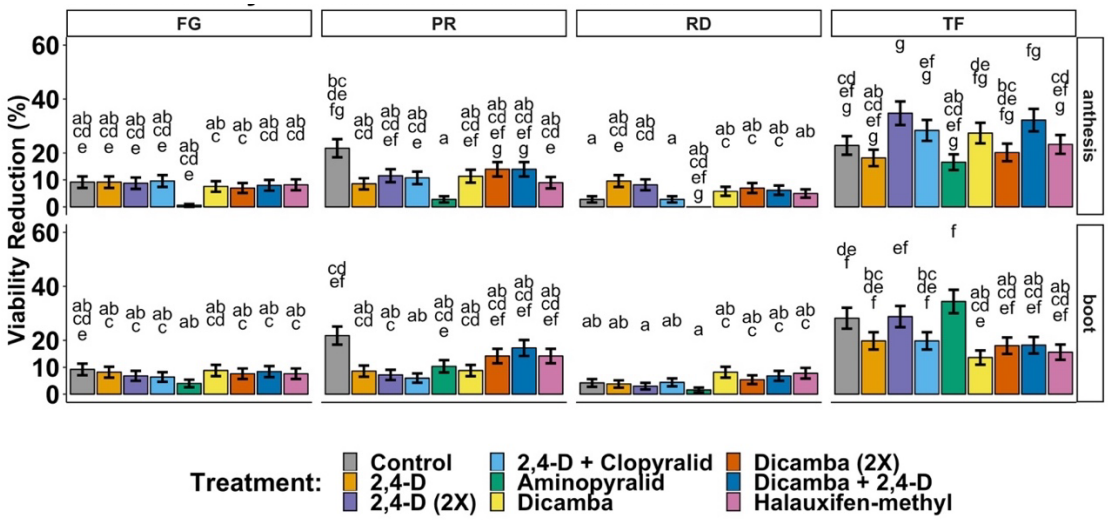



Figure 3-6. Seed viability reduction after accelerated aging test in response of herbicide treatments at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse trial in 2018. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.31

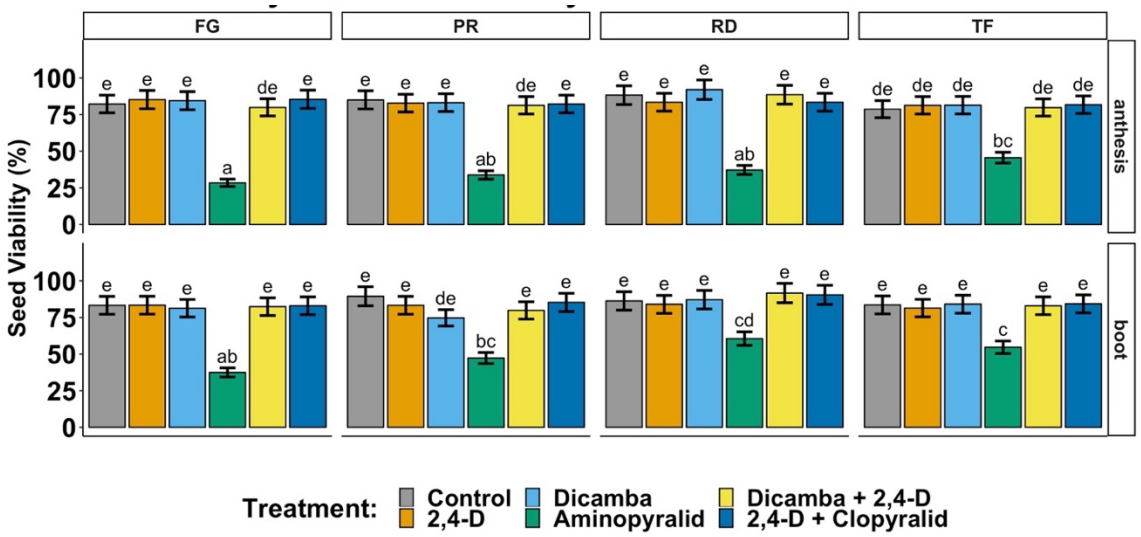



Figure 3-7. Seed viability in response of herbicide treatments at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse trials. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.32

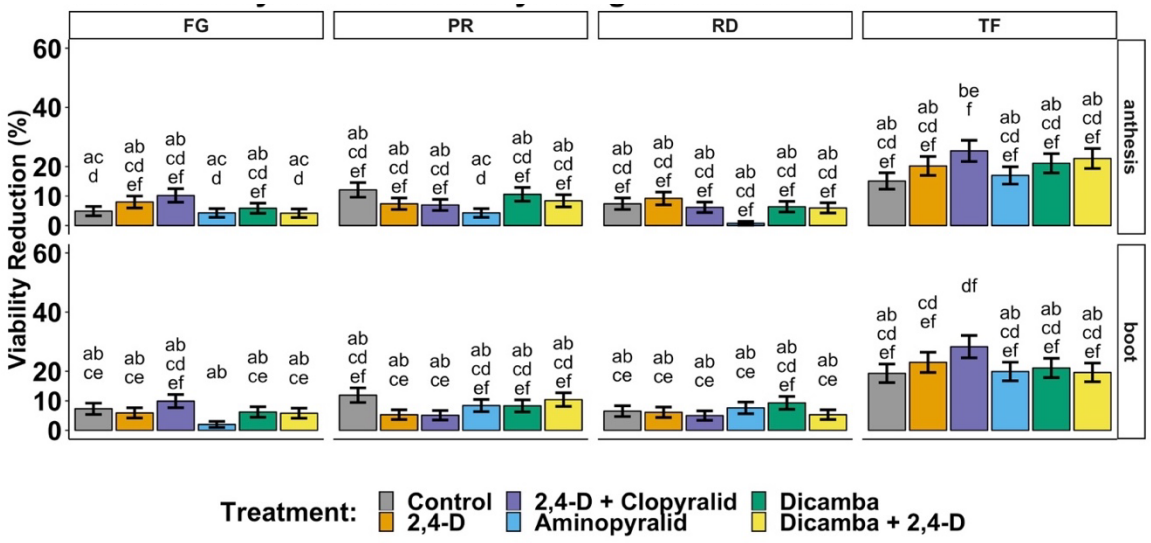



Figure 3-8. Seed viability reduction after accelerated aging test in response of herbicide treatments at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in both greenhouse trials. (Letters represent different groups on HSD Tukey test p-value < 0.05). Means in Table B.33

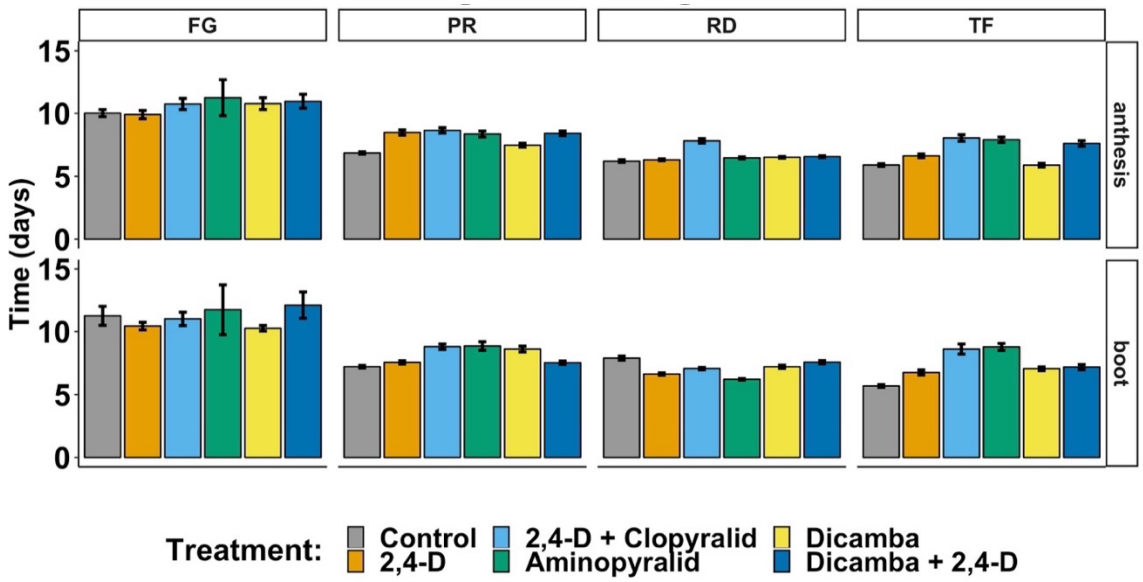



Figure 3-9. Comparison of parameter TD-50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse 2017 trial. Parameters in Table B.34 and Table B.35

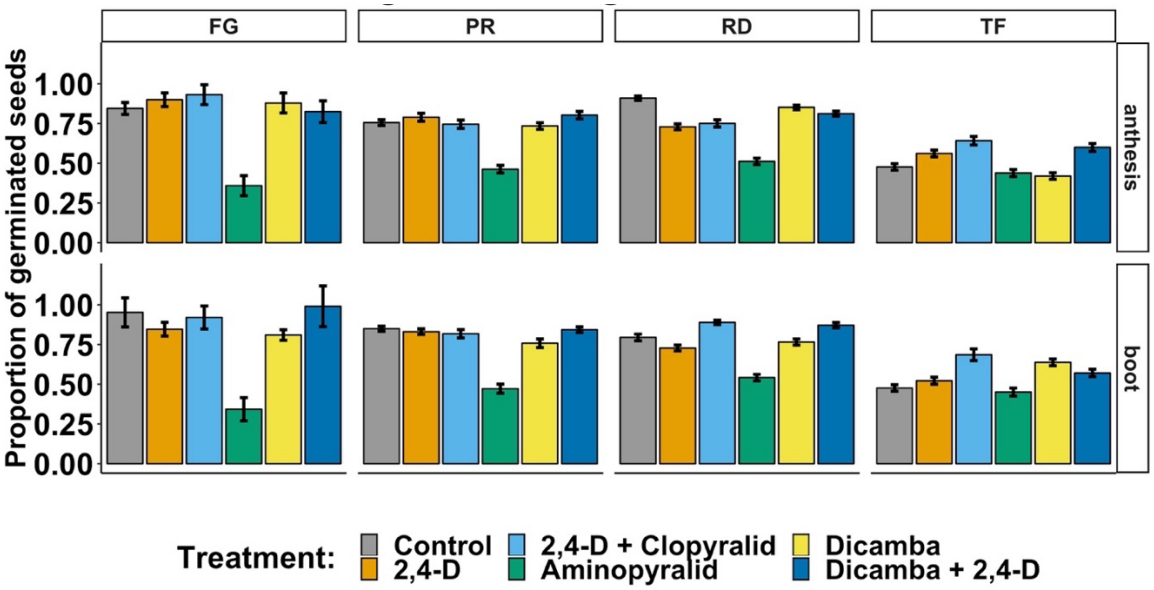



Figure 3-10. Comparison of parameter GermMax (Maximum germination after germination test period) of the fitted three-parameter log-logistic regression showing the difference in maximum of germination at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse 2017 trial. Parameters in Table B.34 and Table B.35

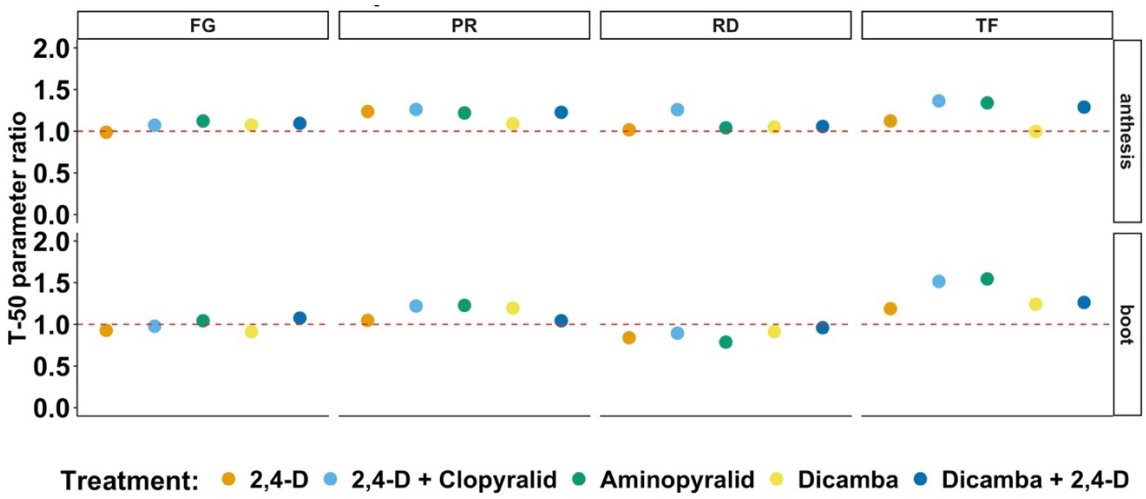



Figure 3-11. Comparison of ratio between treatments and untreated check of parameter TD-50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse 2017 trial. Parameters in Table B.34 and
Table B.35

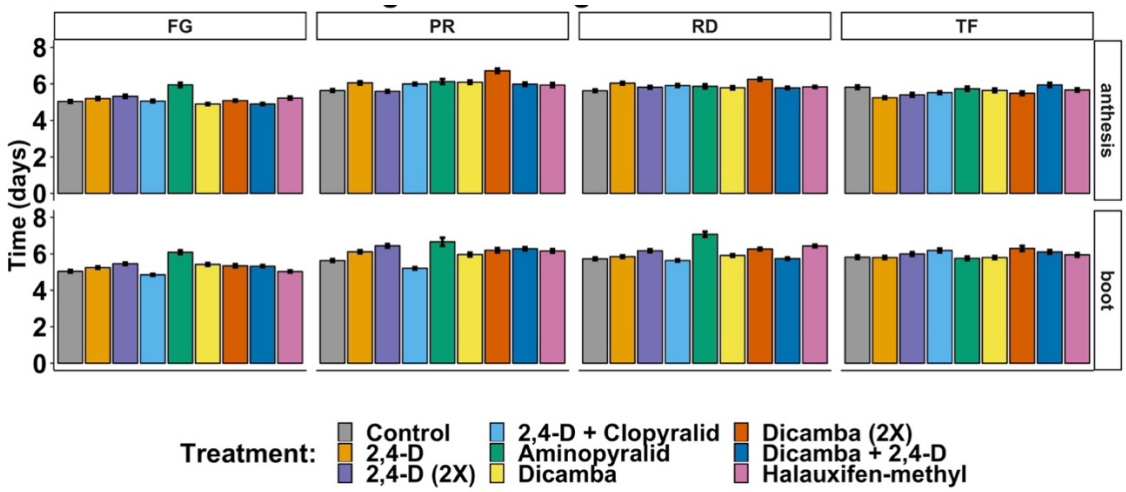



Figure 3-12. Comparison of parameter T50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse 2018 trial. Parameters in Table B.36 and Table B.37

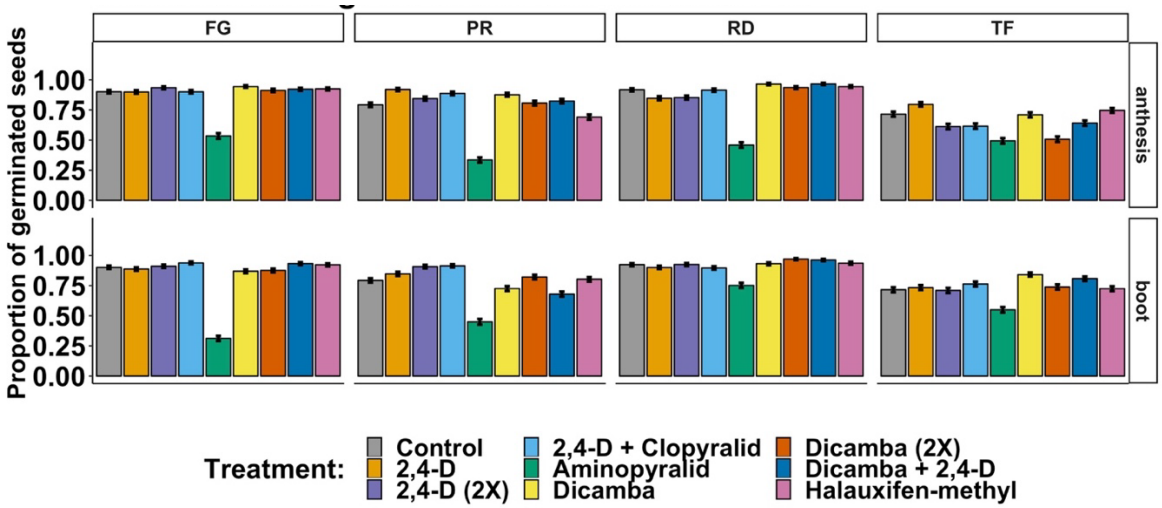



Figure 3-13. Comparison of parameter GermMax (Maximum germination after germination test period) of the fitted three-parameter log-logistic regression showing the difference in maximum of germination at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse 2018 trial. Parameters in Table B.36 and Table B.37

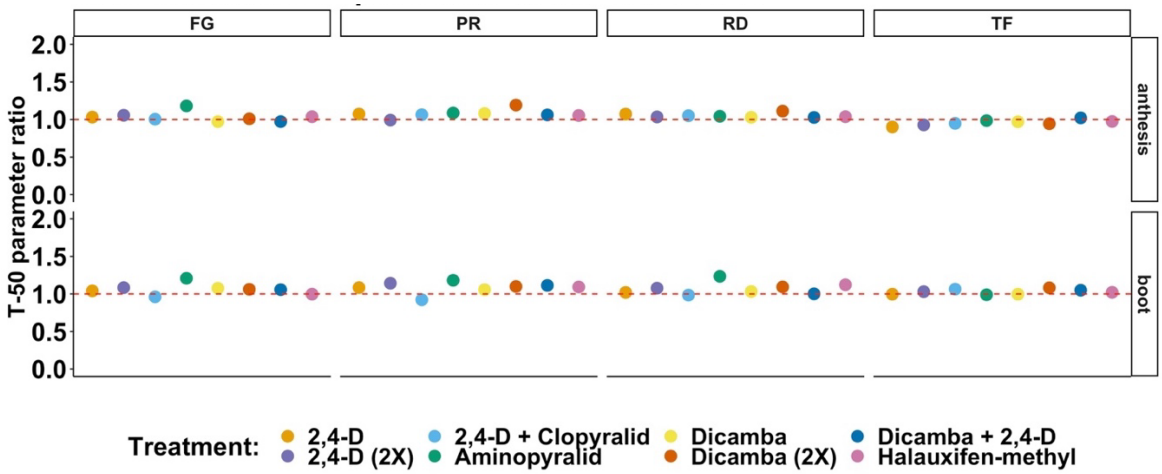



Figure 3-14. Comparison of ratio between treatments and untreated check of parameter TD50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (boot and anthesis) and biotypes (FG, PR, RD and TF) in greenhouse 2018 trial. Parameters in Table B.36 and Table B.37

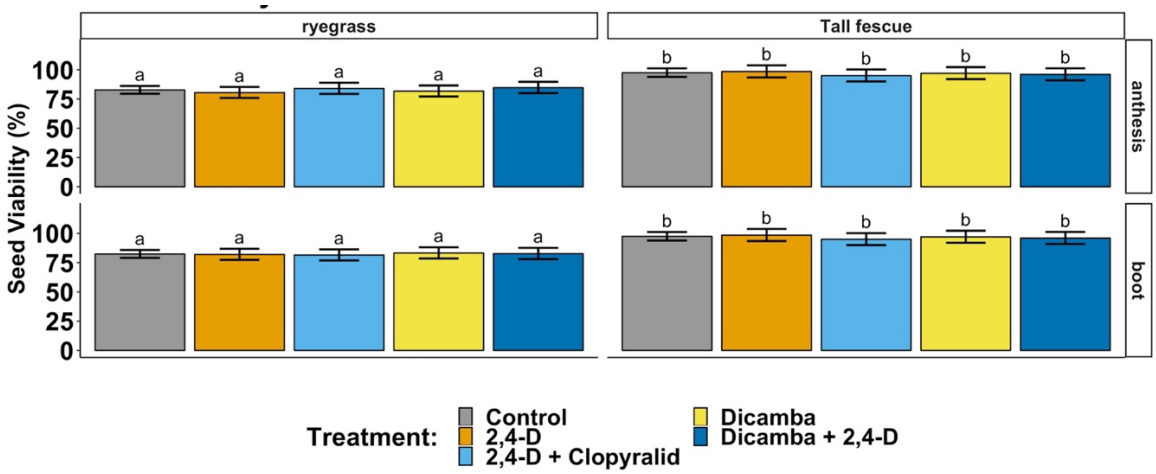



Figure 3-15. Seed viability in response of herbicide treatments at different stages (anthesis and boot) and grass species on Dallas, OR trial. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.38

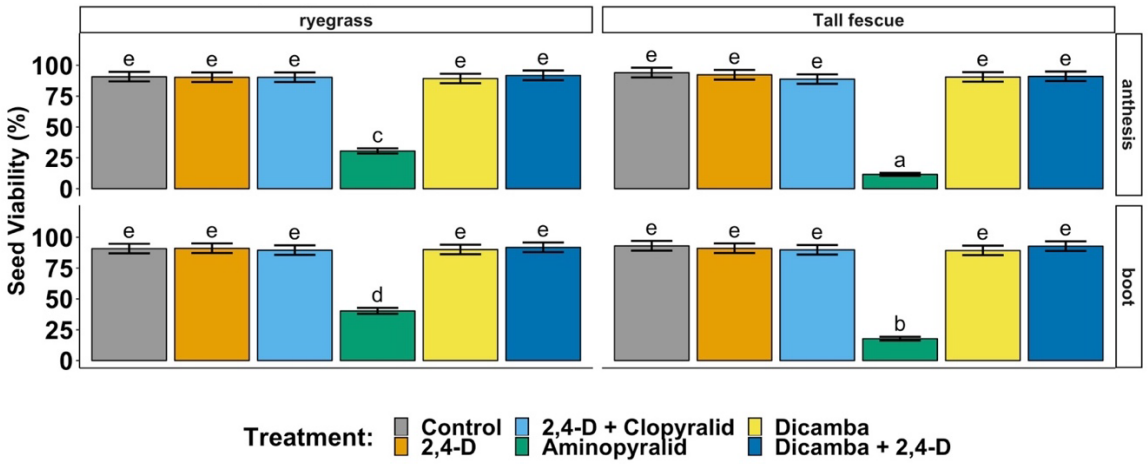



Figure 3-16. Seed viability in response of herbicide treatments at different stages (anthesis and boot) and grass species on Hyslop farm in Corvallis, OR, trial. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.39

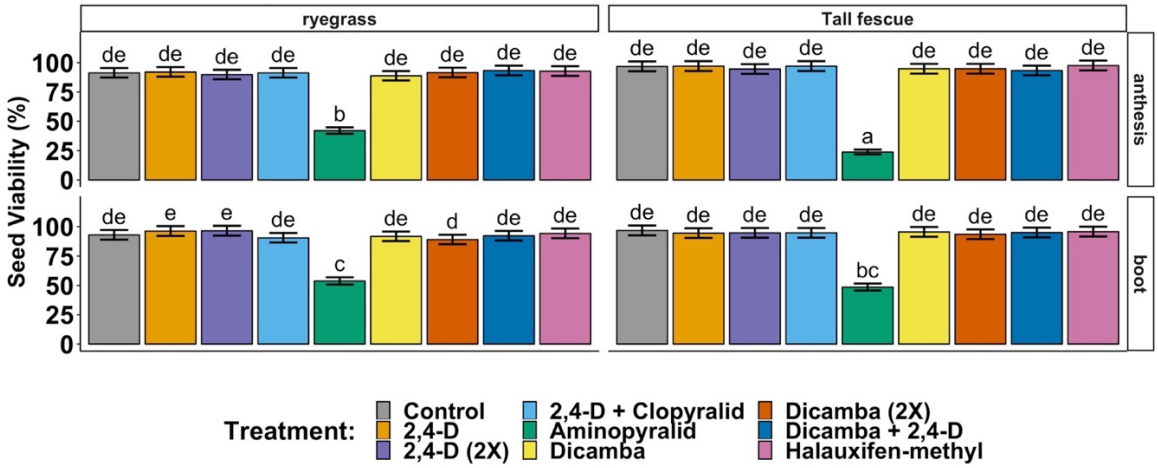



Figure 3-17. Seed viability in response of herbicide treatments at different stages (anthesis and boot) and grass species in Gaston, OR, trial. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.40

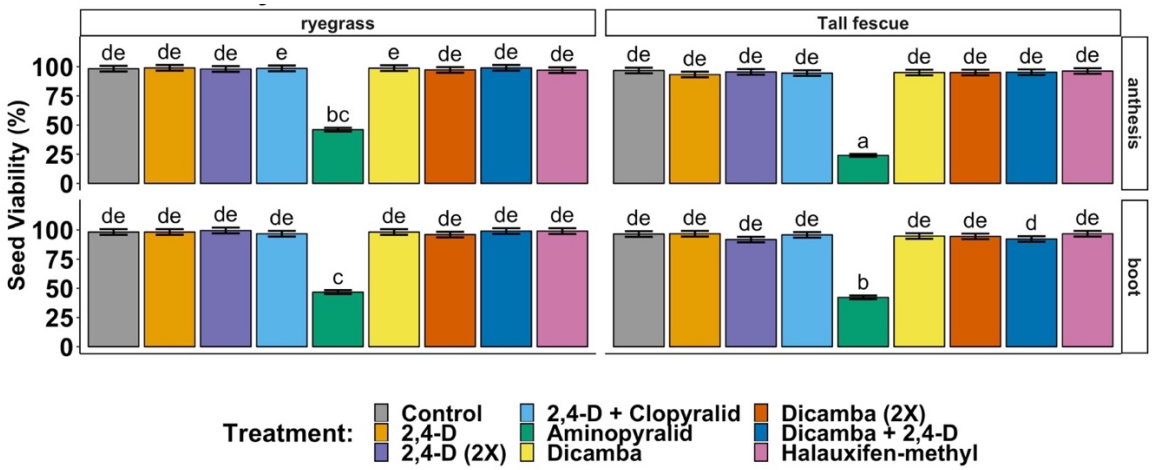



Figure 3-18. Seed viability in response of herbicide treatments at different stages (anthesis and boot) and grass species on Schmidt farm in Corvallis, OR, trial. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.41

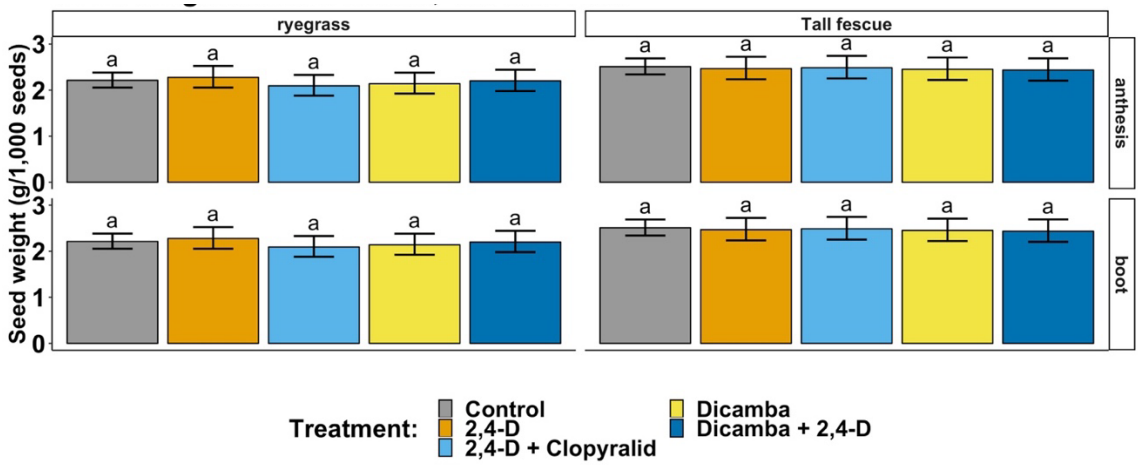



Figure 3-19. Seed weight test results in response of herbicide treatments at different stages (anthesis and boot) and grass species in Dallas, OR, trial. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.42

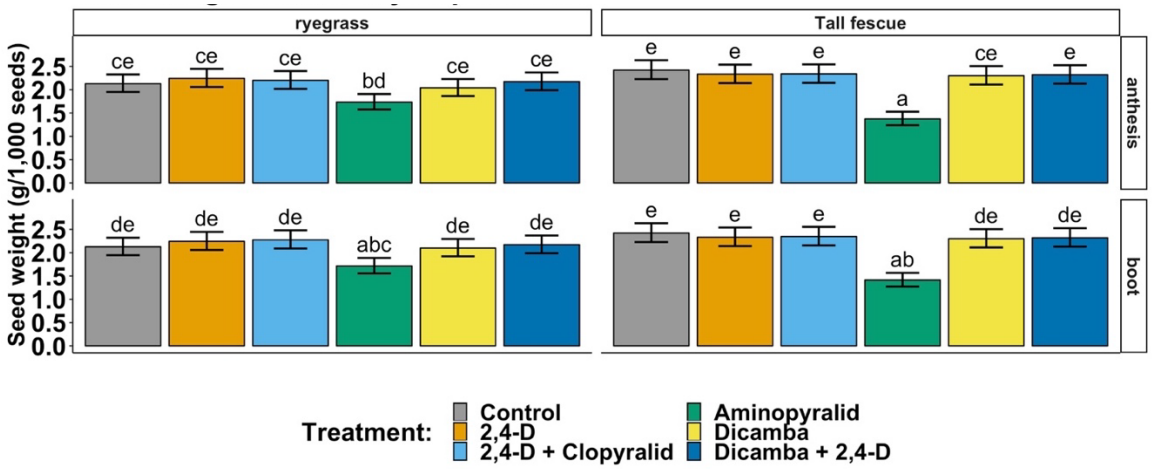



Figure 3-20. Seed weight in response of herbicide treatments at different stages (anthesis and boot) and grass species in Hyslop farm in Corvallis, OR, trial. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.43

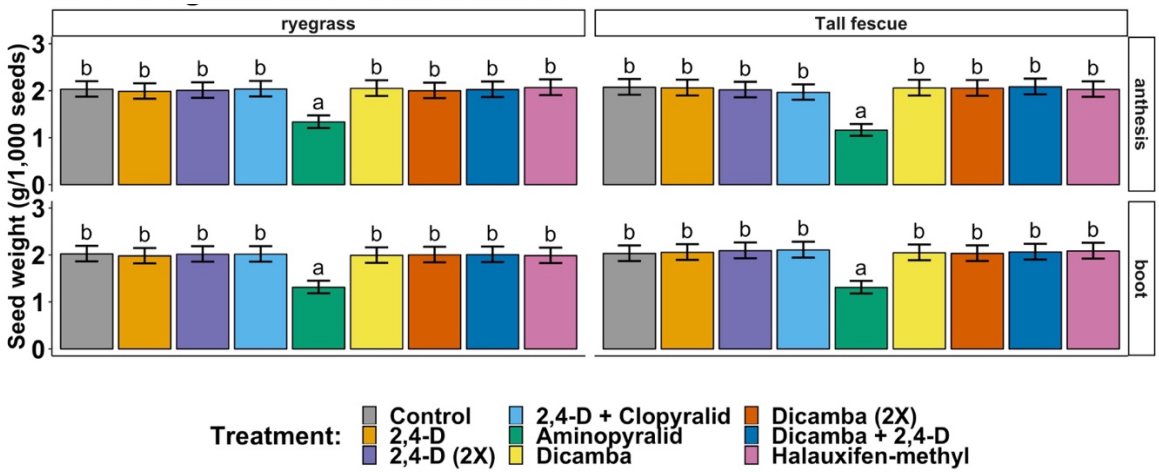



Figure 3-21. Seed weight in response of herbicide treatments at different stages (anthesis and boot) and grass species in Gaston, OR, trial. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.44

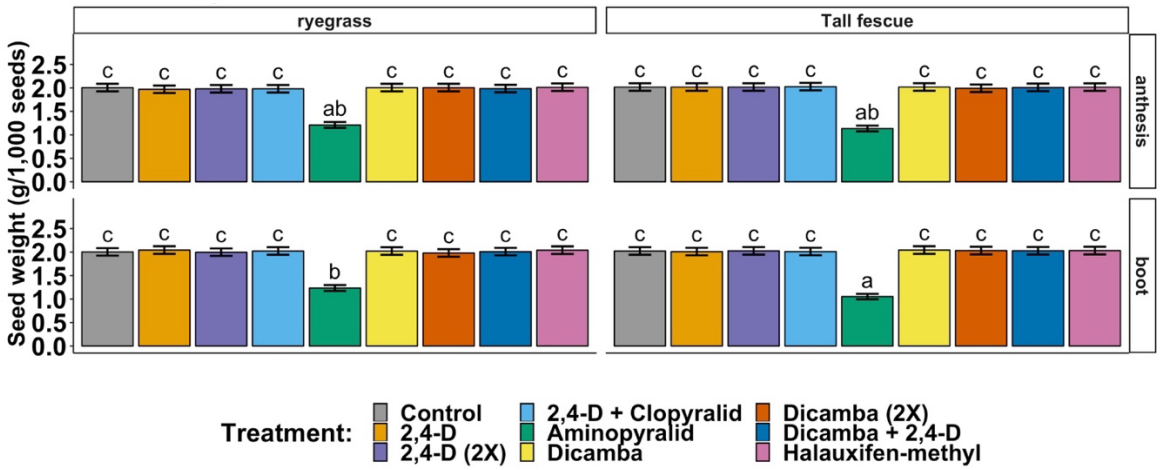



Figure 3-22. Seed weight in response of herbicide treatments at different stages (anthesis and boot) and grass species in Schmidt farm trial. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.45

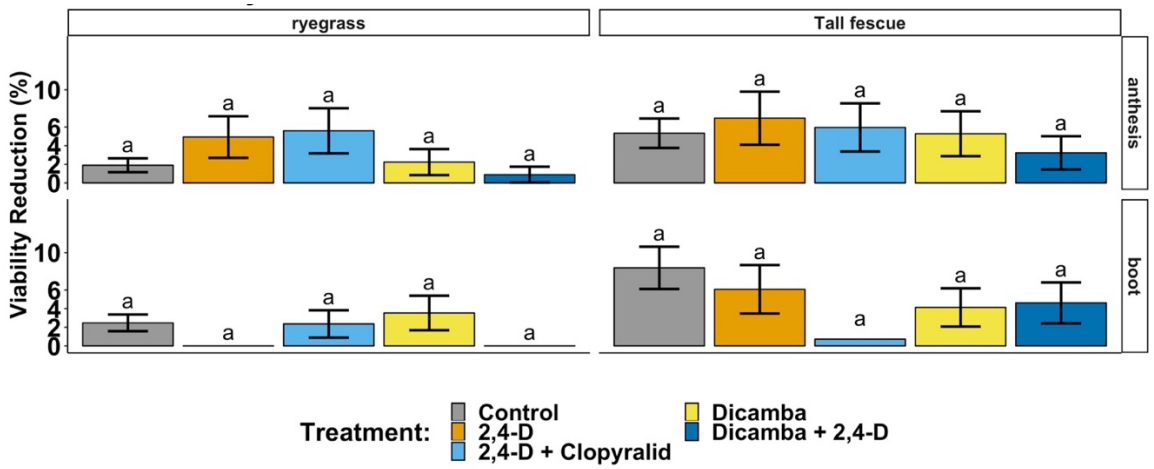



Figure 3-23. Seed viability reduction after accelerated aging test in response of herbicide treatments at different stages (anthesis and boot) and grass species in Dallas, OR, trials. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.46

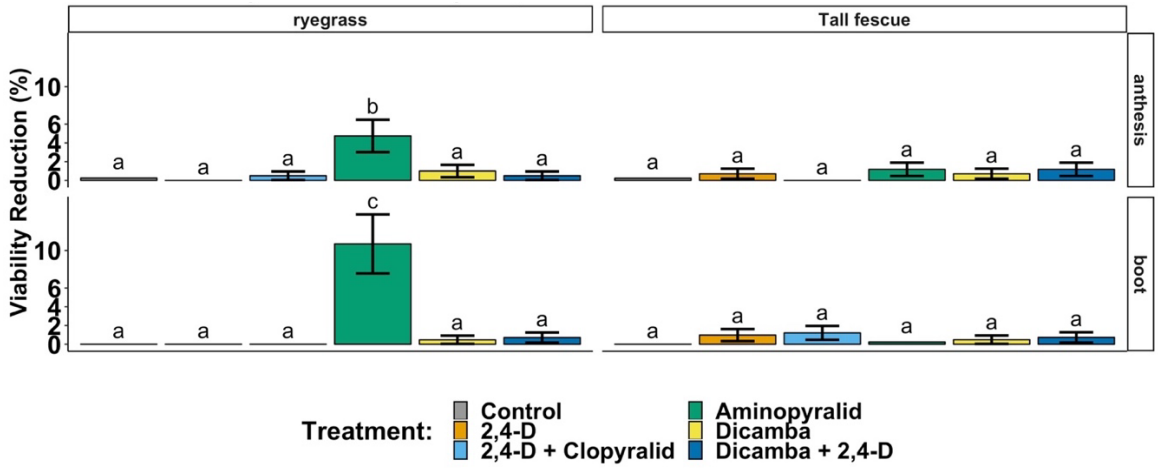



Figure 3-24. Seed viability reduction after accelerated aging test in response of herbicide treatments at different stages (anthesis and boot) and grass species on Hyslop farm on Corvallis, OR, trials. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.47

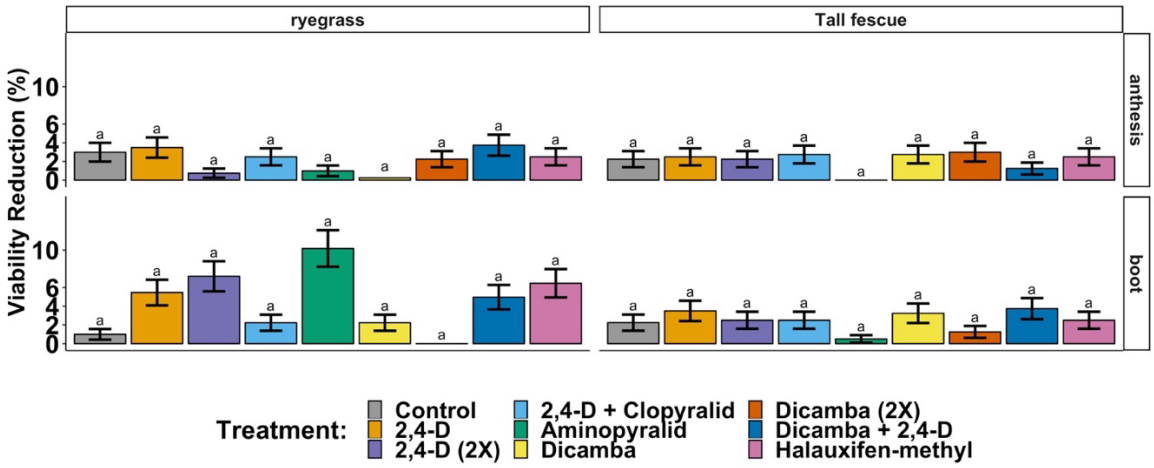



Figure 3-25. Seed viability reduction after accelerated aging test in response of herbicide treatments at different stages (anthesis and boot) and grass species in Gaston, OR, trials. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.48

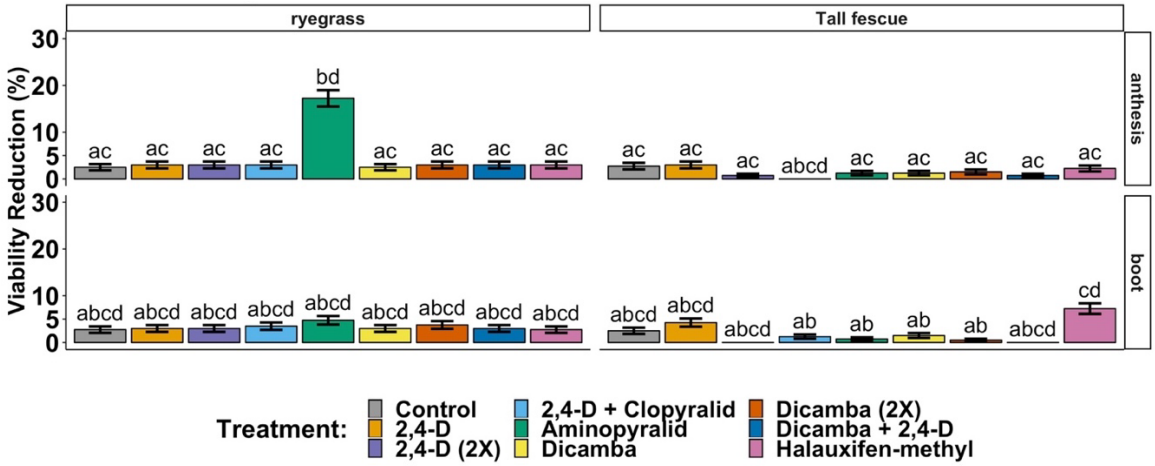



Figure 3-26. Seed viability reduction after accelerated aging test in response of herbicide treatments at different stages (anthesis and boot) and grass species in Schmidt farm in Corvallis, OR, trials. (Letters represent different mean groups on HSD Tukey test p-value < 0.05). Means in Table B.49

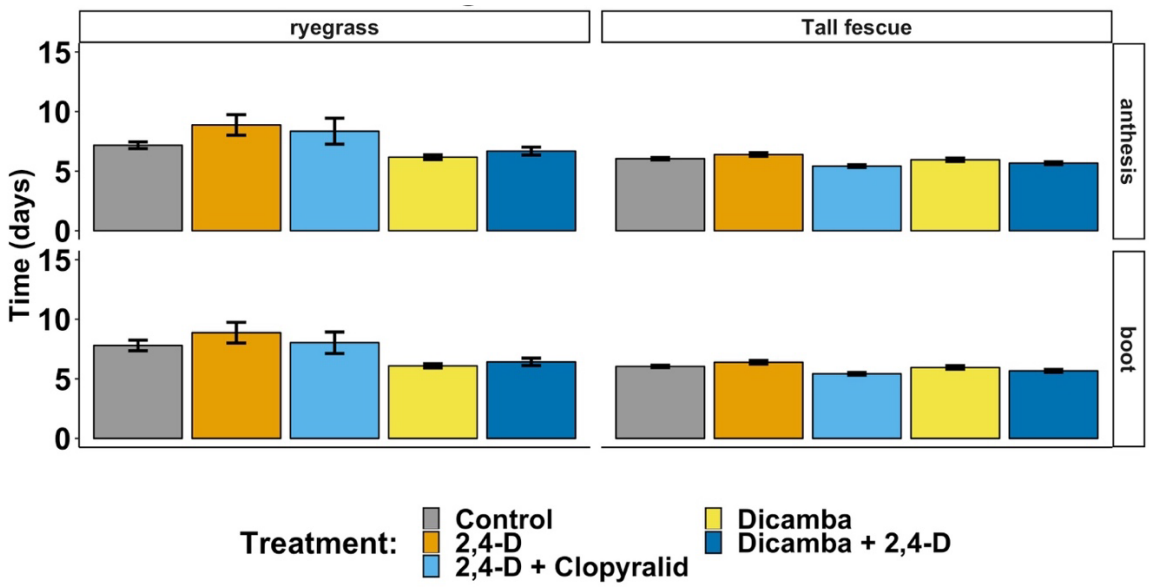



Figure 3-27. Comparison of parameter T50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (anthesis and boot) and grass species in Dallas, OR, trial. Parameters in Table B.50

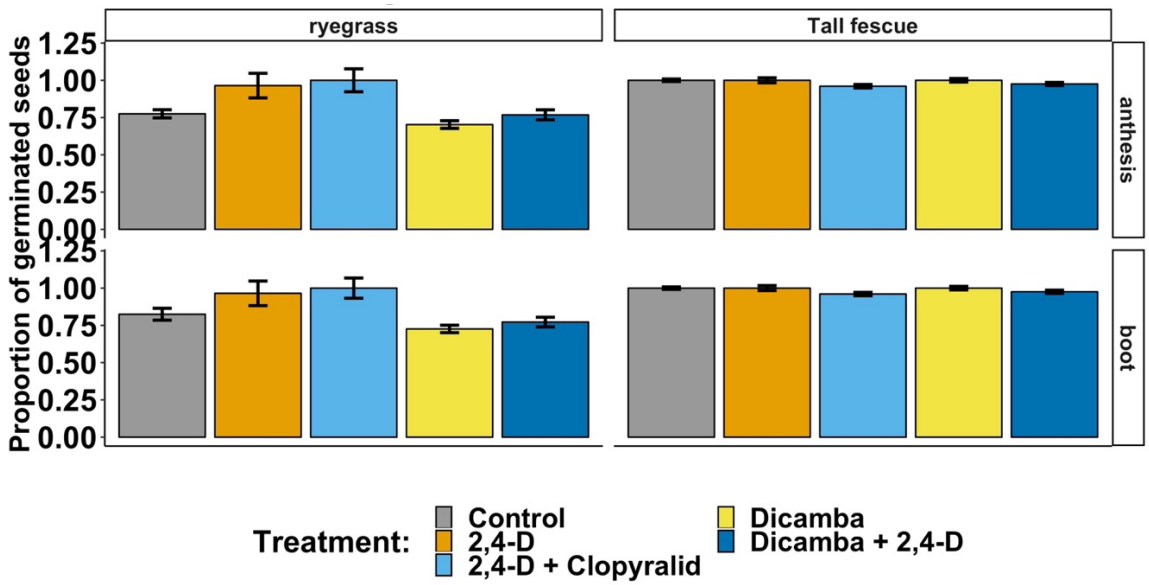



Figure 3-28. Comparison of parameter GermMax (Maximum germination after germination test period) of the fitted three-parameter log-logistic regression showing the difference in maximum of germination at different stages (anthesis and boot) and grass species in Dallas, OR, trial. Parameter in Table B.50

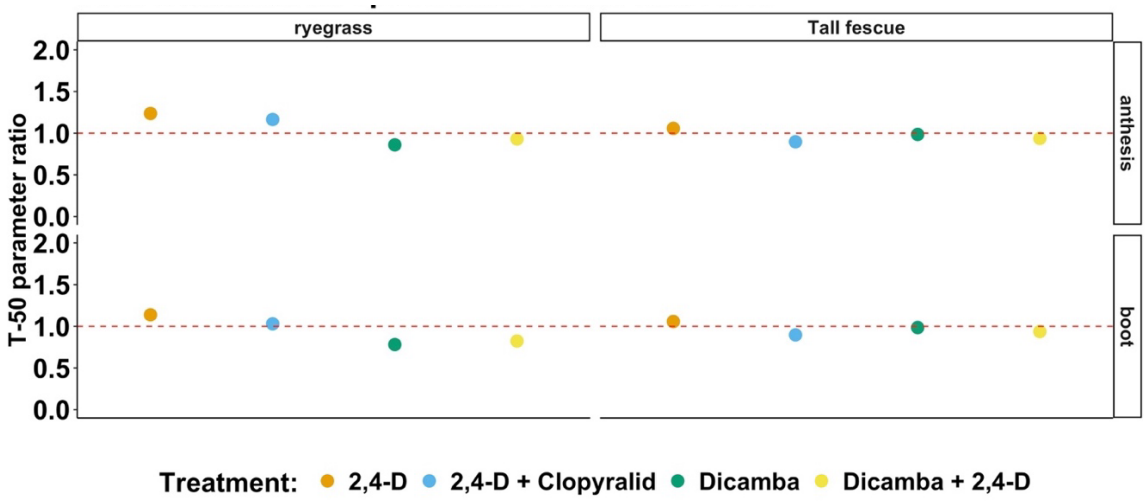



Figure 3-29. Comparison of ratio between treatments and untreated check of parameter TD50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (anthesis and boot) and grass species in Dallas, OR, trial. Parameter in Table B.50

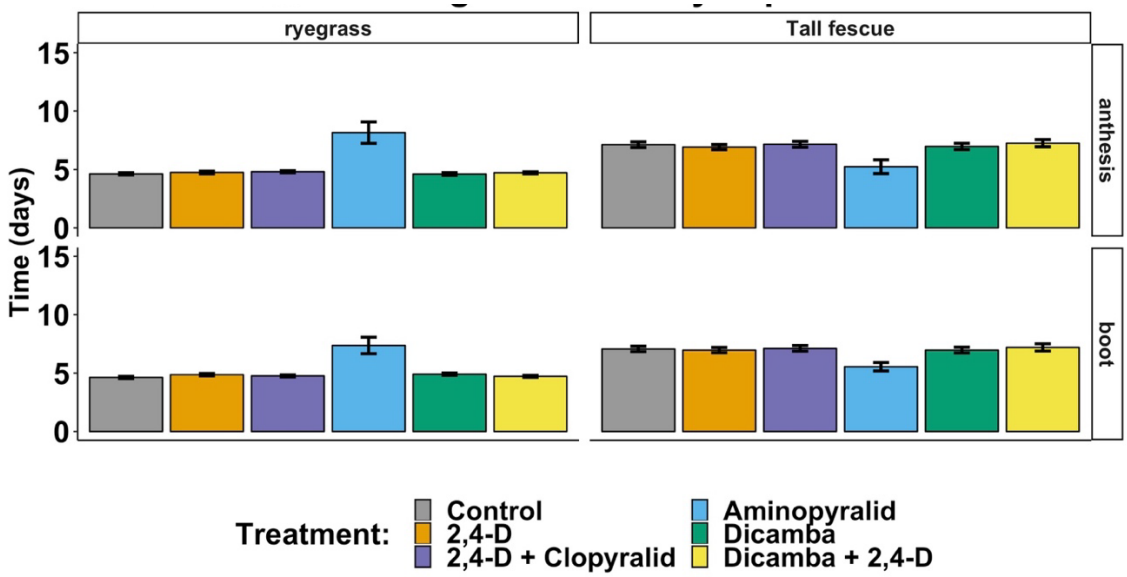



Figure 3-30. Comparison of parameter T50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (anthesis and boot) and grass species in Hyslop farm Corvallis, OR, trial. Parameter in Table B.51

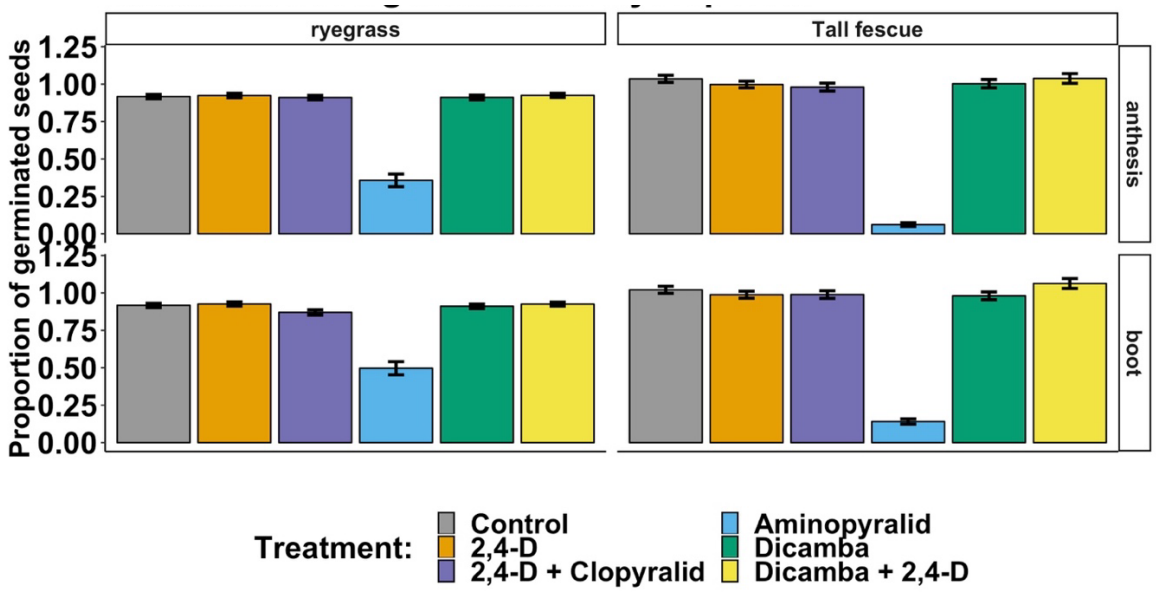



Figure 3-31. Comparison of parameter GermMax (Maximum germination after germination test period) of the fitted three-parameter log-logistic regression showing the difference in maximum of germination at different stages (anthesis and boot) and grass species in Hyslop farm Corvallis, OR, trial. Parameter in Table B.51

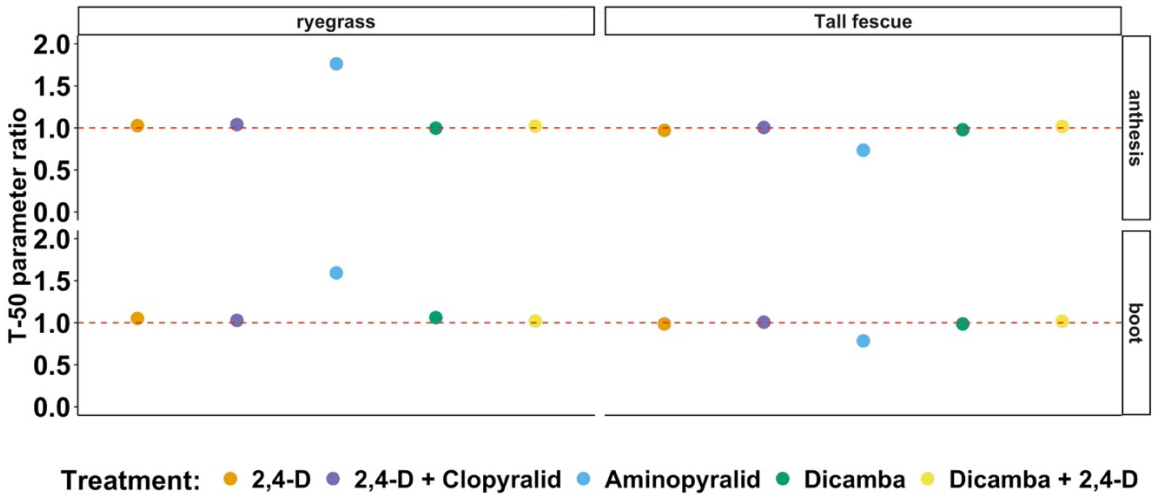



Figure 3-32. Comparison of ratio between treatments and untreated check of parameter TD50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (anthesis and boot) and grass species in Hyslop farm Corvallis, OR, trial. Parameter in Table B.51

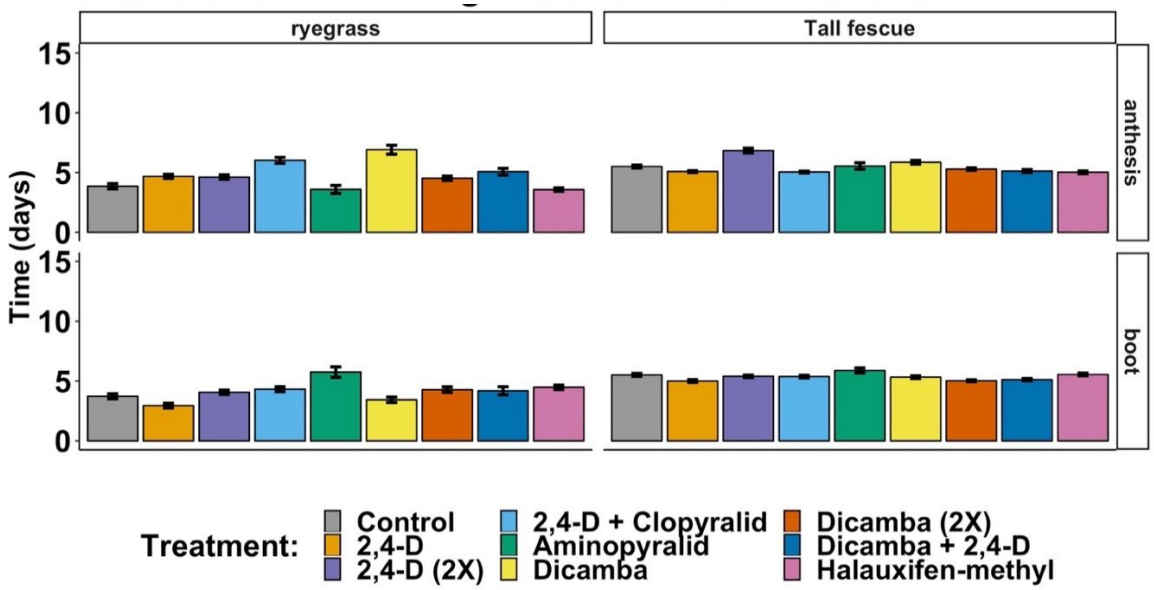



Figure 3-33. Comparison of parameter T50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (anthesis and boot) and grass species in Gaston, OR, 2018 trial. Parameter in Table B.52

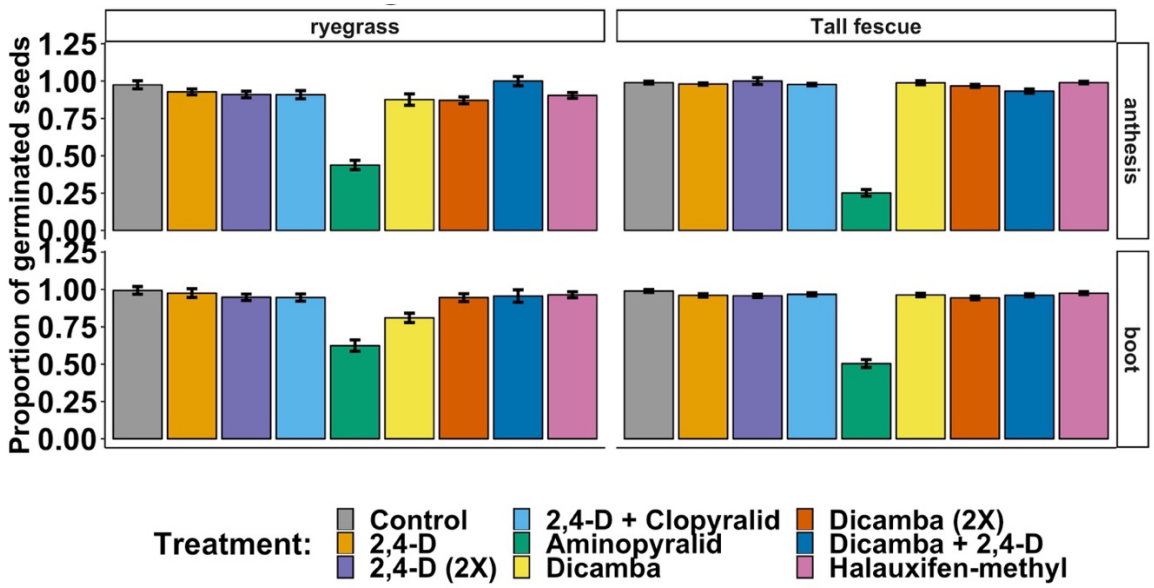



Figure 3-34. Comparison of parameter GermMax (Maximum germination after germination test period) of the fitted three-parameter log-logistic regression showing the difference in maximum of germination at different stages (anthesis and boot) and grass species in Gaston, OR, 2018 trial. Parameter in Table B.52

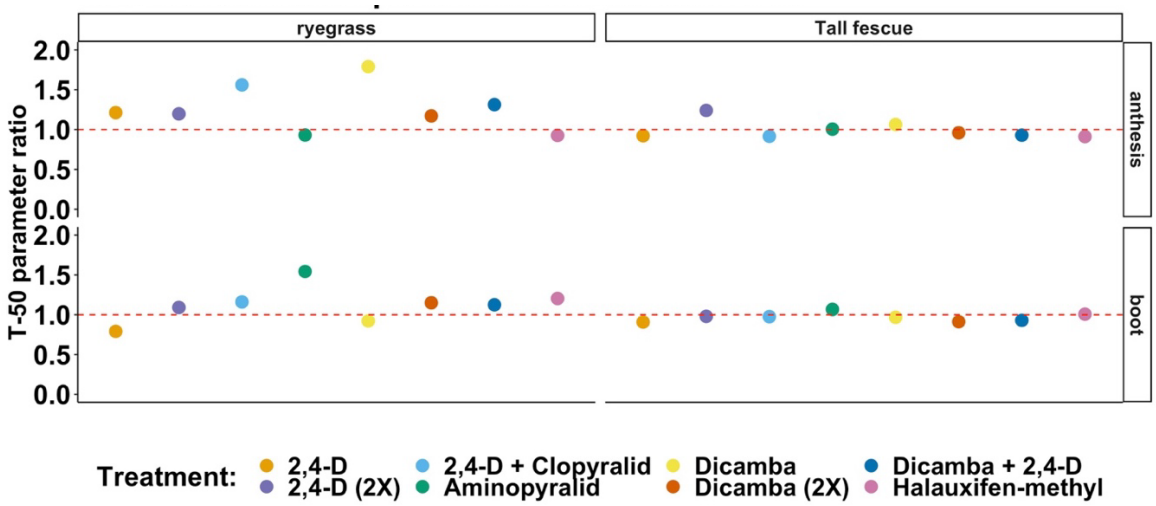



Figure 3-35. Comparison of ratio between treatments and untreated check of parameter TD50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (anthesis and boot) and grass species in Gaston, OR, 2018 trial. Parameter in Table B.52

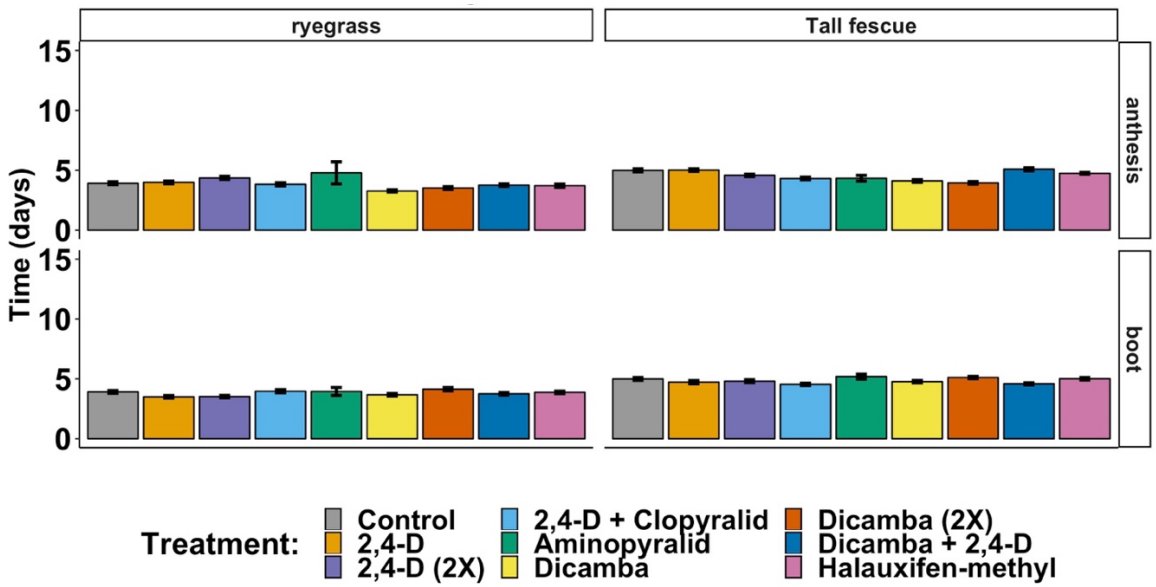



Figure 3-36. Comparison of parameter T50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination for Schmith farm Corvallis, OR, 2018 trial. Parameter in Table B.53

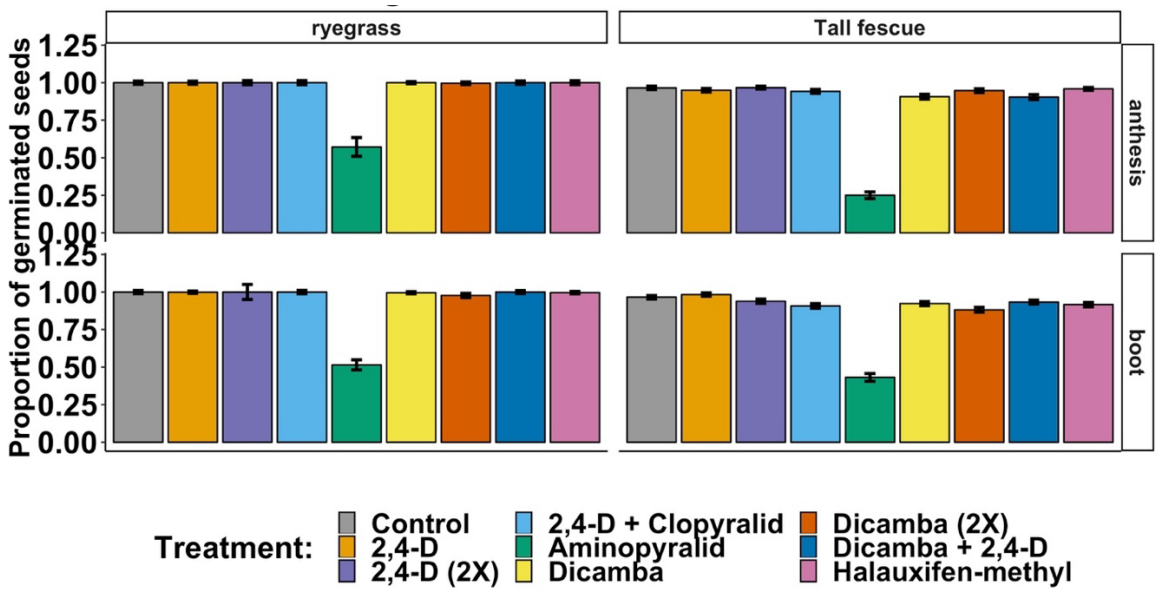



Figure 3-37. Comparison of parameter GermMax (Maximum germination after germination test period) of the fitted three-parameter log-logistic regression showing the difference in maximum of germination at different stages (anthesis and boot) and grass species in Schmidt farm Corvallis, OR, 2018 trial. Parameter in Table B.53

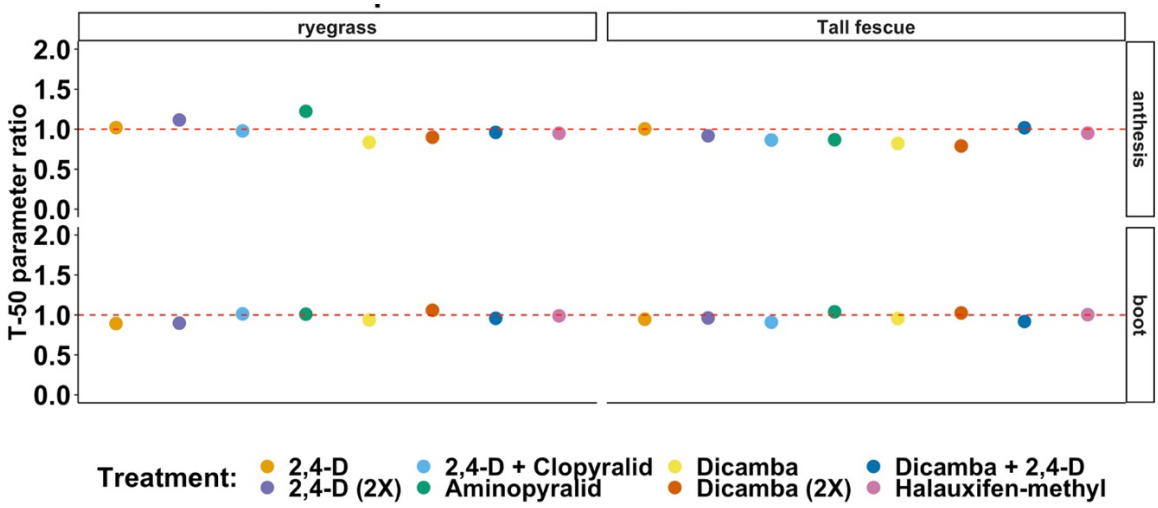



Figure 3-38. Comparison of ratio between treatments and untreated check of parameter TD50 (time to 50% of the seeds germinate) of the fitted three-parameter log-logistic regression showing the difference in speed of germination at different stages (anthesis and boot) and grass species in Gaston, OR, 2018 trial. Parameter in Table B.53

# Tables

Table 3-1. Synthetic auxin herbicides treatments used on field and greenhouse trials

| Treatment | Herbicide | chemical family | Trade name | Company | Rate (kg a.e/ha) |
|---|---|---|---|---|---|
| 1 | Dicamba acid | Benzoates | Vision | Helena Agri-Enterprise | 1.06 |
| 2 | 2,4-D acid | Phenoxy-carboxylates | Unison | Helena Agri-Enterprise | 1.10 |
| 3 | Aminopyralid | Pyridine-carboxtlates | Milestone | Corteva AgroSciences | 0.50 |
| 4 | Dicamba + 2,4-D | Phenoxy-carboxylates + Benzoates | Latigo | Helena Agri-Enterprise | 1.18 |
| 5 | 2,4-D + Clopyralid | Phenoxy + Pyridine carboxylates | Unison + Stinger | Helena + Corteva | 1.10 + 0.28 |
| 6 [a] | Dicamba acid | Benzoates | Vision | Helena Agri-Enterprise | 2.24 |
| 7 [a] | 2,4-D acid | Phenoxy-carboxylates | Unison | Helena Agri-Enterprise | 2.24 |
| 8[a] | Florasulam + Halauxifen-methyl | Arylpicolinates | Quelex | Corteva AgroSciences | 0.84 |

a Treatments applied only on 2018 trials

# CHAPTER 4: General Conclusions

Survey and greenhouse and field trials were conducted to understand the frequency, distribution, ploidy diversity of populations of Italian Ryegrass (*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot) in western Oregon. Control options using synthetic auxin herbicides of for managing Italian ryegrass in tall fescue seed production were tested.

This was the first formal field survey on herbicide resistance and ploidy diversity in Italian ryegrass in western Oregon. From the 150 fields sampled, 50% of the fields had Italian ryegrass present. These results indicate a lack of effective control in the agricultural fields surveyed and that Italian ryegrass was confirmed as one of the most troublesome weeds in the Willamette Valley. Results also pointed to a higher chance of finding Italian ryegrass infestations in wheat fields when compared to tall fescue fields indicating a possible intense selection pressure on the management of this weed in this specific crop. It is necessary to evaluate what crops wheat is been rotated with to check the effects of it on the presence of Italian ryegrass.

Similar to previous global research, this survey indicates a worrying scenario regarding the frequency of herbicide resistance. From the tested populations, 88% showed resistance presence to at least one herbicide. This high frequency is likely due to the repetitive types of herbicides and modes of action being used over years, indicating the need for new management approaches to control the spread of resistance.

The most common types of resistance were to Acetyl-CoA carboxylase (ACCase), acetolactate synthase (ALS) and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPs) inhibitors. Multiple-resistance with combinations of these modes of action was also

frequent among the tested populations. Multiple resistance frequency in the tested populations was of 75% (56 populations). For tall fescue fields where Italian ryegrass was present, there was a frequency of 55% (22 populations) of multiple resistance while for the wheat fields 94% (17 populations) of the populations had both multiple and cross-resistance.

These results will serve as a basis for future studies to understand the presence of resistance and to create new strategies to minimize the spread of it. This survey should be conducted for additional years to create a multi-year analysis to understand the evolution and spread of resistance. Other species should be added to the survey.

Greenhouse and multiple field trials were conducted in 2017 and 2018 to assess the effects of synthetic auxin herbicides on seed viability of Italian ryegrass. Herbicides were sprayed late in the season to test as a possible management tool to minimize seed viability of Italian ryegrass plants. Of the eight herbicides tested, aminopyralid was the only one that affected seed quality.

In the greenhouse trials, four biotypes with resistance to different mode of action were tested, and some differences were found among the populations. This variation in response to aminopyralid, indicates that an optimum herbicide rate is still needs to be determined to reduce seed viability of multiple Italian ryegrass biotypes. Aminopyralid tested in field trials also reduced seed quality of Italian ryegrass. However, the effects over tall fescue were more substantial than on the target weed, making this management method unfeasible in tall fescue seed production. Because aminopyralid was the only herbicide showing effects, further studies are needed to understand the molecular aspects response of aminopyralid on seed development of Italian ryegrass. Future studies should

evaluate crop safety for aminopyralid application in crops such as perennial orchard crops.

## Bibliography

1. Fearon C, Hayward M, Lawrence M. Self-incompatibility in ryegrass. *Heredity*. **50**:35–45 (1983).

2. Karn E, Jasieniuk M. Genetic diversity and structure of *Lolium perenne* ssp. *multiflorum* in California vineyards and orchards indicate potential for spread of herbicide resistance via gene flow. *Evolutionary Applications*. **10**:616–29 (2017).

3. Giddings G. Modelling the spread of pollen from *Lolium perenne*. The implications for the release of wind-pollinated transgenics. *Theoretical and Applied Genetics*. **100**:971–4 (2000).

4. Giddings G, Hamilton NS, Hayward M. The release of genetically modified grasses. Part 1: pollen dispersal to traps in *Lolium perenne*. *Theoretical and Applied Genetics*. **94**:1000–6 (1997).

5. Meyers SC. Flora of Oregon. Volume 1, Pteridophytes, gymnosperms, and monocots. 1st ed. Vol. 1. Fort Worth, Texas: Botanical Research institute; pp. 591 (2015).

6. Kopecký D, Loureiro J, Zwierzykowski Z, Ghesquière M, Doležel J. Genome constitution and evolution in *Lolium* × *Festuca* hybrid cultivars (*Festulolium*). *Theor Appl Genet*. **113**:731–42 (2006).

7. Lewis E. *Festuca* L. x *Lolium* L.= *Festulolium* Aschers and Graebn. *Stace CA (ed) Hybridization and the Xora of the British Isles*. :547–52 (1975).

8. Lamote V, Baert J, Roldán-Ruiz I, De Loose M, Van Bockstaele E. Tracing of 2n egg occurrence in perennial ryegrass (*Lolium perenne* L.) using interploidy crosses. *Euphytica*. **123**:159–64 (2002).

9. Pyšek P, Skálová H, Čuda J, Guo W-Y, Suda J, Doležal J, et al. Small genome separates native and invasive populations in an ecologically important cosmopolitan grass. *Ecology*. **99**:79–90 (2018).

10. Gibbs G, Watson L, Koekemoer M, Smook L, Barker N, Anderson H, et al. Grasses of southern Africa. Memoirs of the botanical survey of South Africa. 2nd ed. Vol. 58. National Botanic Gardens, Botanical Research Institute; pp. 437 (1990).

11. Bock DG, Kantar MB, Caseys C, Matthey-Doret R, Rieseberg LH. Evolution of invasiveness by genetic accommodation. *Nature Ecology & Evolution*. **2**:991 (2018).

12. Powles SB, Yu Q. Evolution in action: plants resistant to herbicides. *Annual review of plant biology*. **61**:317–47 (2010).

13. Busi R, Powles SB. Evolution of glyphosate resistance in a *Lolium rigidum* population by glyphosate selection at sublethal doses. *Heredity*. **103**:318–25 (2009).

14. Olofsson SK, Cars O. Optimizing drug exposure to minimize selection of antibiotic resistance. *Clinical Infectious Diseases*. **45**:129–36 (2007).

15. Roush RT, McKenzie JA. Ecological genetics of insecticide and acaricide resistance. *Annual review of entomology*. **32**:361–80 (1987).

16. Shaw M. Is there such a thing as a fungicide resistance strategy? A modeller's perspective. *Aspects of Applied Biology*. **78**:37 (2006).

17. Brunharo CA, Hanson BD. Multiple herbicide–resistant Italian ryegrass [*Lolium perenne* L. spp. *multiflorum* (Lam.) Husnot] in California perennial crops: characterization, mechanism of resistance, and chemical management. *Weed Science*. **66**:696–701 (2018).

18. Délye C, Jasieniuk M, Le Corre V. Deciphering the evolution of herbicide resistance in weeds. *Trends in Genetics*. **29**:649–58 (2013).

19. Duke SO. Why have no new herbicide modes of action appeared in recent years? *Pest Manag Sci*. **68**:505–12 (2012).

20. Avila-Garcia WV, Mallory-Smith C. Glyphosate-resistant Italian ryegrass (*Lolium perenne*) populations also exhibit resistance to glufosinate. *Weed science*. **59**:305–309 (2011).

21. Llewellyn RS, Powles SB. High levels of herbicide resistance in rigid ryegrass (*Lolium rigidum*) in the wheat belt of Western Australia. *Weed Technology*. **15**:242–248 (2001).

22. Bararpour MT, Norsworthy JK, Burgos NR, Korres NE, Gbur EE. Identification and biological characteristics of ryegrass (*Lolium* spp.) accessions in Arkansas. *Weed Science*. **65**:350–60 (2017).

23. Betts KJ, Ehlke NJ, Wyse DL, Gronwald JW, Somers DA. Mechanism of inheritance of diclofop resistance in Italian ryegrass (*Lolium multiflorum*). *Weed Science*. **40**:184–189 (1992).

24. Owen MJ, Martinez NJ, Powles SB. Multiple herbicide-resistant *Lolium rigidum* (annual ryegrass) now dominates across the Western Australian grain belt. *Weed Research*. **54**:314–324 (2014).

25. Karn E, Beffa R, Jasieniuk M. Variation in response and resistance to glyphosate and glufosinate in California populations of Italian ryegrass (*Lolium perenne* ssp. *multiflorum*). *Weed Science*. **66**:168–79 (2018).

26. Powles SB, Lorraine-Colwill DF, Dellow JJ, Preston C. Evolved resistance to glyphosate in rigid ryegrass (*Lolium rigidum*) in Australia. *Weed science*. **46**:604–7 (1998).

27. Ellstrand NC. Current knowledge of gene flow in plants: implications for transgene flow. *Philosophical Transactions: Biological Sciences*. **358**:1163–70 (2003).

28. Levin DA, Kerster HW. Gene flow in seed plants. In: *Gene flow in seed plants*. Springer; p.139–220 (1974).

29. Stebbins GL. Variation and evolution in plants: progress during the past twenty years. In: *Essays in evolution and genetics in honor of Theodosius Dobzhansky*. Boston, MA: Springer; p.173–208 (1970).

30. Ellstrand NC, Rieseberg LH. When gene flow really matters: gene flow in applied evolutionary biology. *Evolutionary Applications*. **9**:833–6 (2016).

31. Dewitt N. Gene flow from crops to weeds. *Nature Biotechnology*. **17**:318 (1999).

32. Feldman M, Sears ER. The wild gene resources of wheat. *Scientific American*. **244**:102–13 (1981).

33. Martins BAB, Leonard JM, Sun L, Zemetra RS, Mallory-Smith C. Selection pressure effects on the proportion and movement of resistance alleles introgressed from wheat in *Aegilops cylindrica*. *Weed Research*. **56**:293–303 (2016).

34. Ellstrand NC, Prentice HC, Hancock JF. Gene flow and introgression from domesticated plants into their wild relatives. *Annual review of Ecology and Systematics*. **30**:539–63 (1999).

35. Henderson IR, Salt DE. Natural genetic variation and hybridization in plants. *Journal of experimental botany*. **68**:5415 (2017).

36. Warwick SI. Gene flow between GM crops and related species in Canada. *The First Decade of Herbicide Resistant Crops in Canada Topics in Canadian Weed Science*. **4**:101–13 (2007).

37. Zapiola M, Campbell C, Butler M, Mallory-Smith C. Escape and establishment of transgenic glyphosate-resistant creeping bentgrass (*Agrostis stolonifera*) in Oregon, USA: a 4-year study. *Journal of Applied Ecology*. **45**:486–94 (2008).

38. Welles SR, Ellstrand NC. Genetic structure reveals a history of multiple independent origins followed by admixture in the allopolyploid weed *Salsola ryanii*. *Evolutionary applications*. **9**:871–8 (2016).

39. Sarangi D, Tyre AJ, Patterson EL, Gaines TA, Irmak S, Knezevic SZ, et al. Pollen-mediated gene flow from glyphosate-resistant common waterhemp (*Amaranthus rudis*

Sauer): consequences for the dispersal of resistance genes. *Scientific Reports*. **7**:44913 (2017).

40. Wit F. Natural and experimental hybrids of ryegrasses and meadow fescue. *Euphytica*. **13**:294–304 (1964).

41. Pašakinskienė I, Anamthawat-Jonsson K, Humphreys M, Paplauskiene V, Jones R. New molecular evidence on genome relationships and chromosome identification in fescue (*Festuca*) and ryegrass (*Lolium*). *Heredity*. **81**:659 (1998).

42. Akiyama Y, Ueyama Y, Hamada S, Kubota A, Kato D, Yamada-Akiyama H, et al. Utilization of flow cytometry for *festulolium* breeding (*Lolium multiflorum* (2x)× *Festuca arundinacea* (6x)). *Breeding science*. **66**:234–43 (2016).

43. Hüsken A, Dietz-Pfeilstetter A. Pollen-mediated intraspecific gene flow from herbicide resistant oilseed rape (*Brassica napus* L.). *Transgenic research*. **16**:557–69 (2007).

44. Millwood R, Nageswara-Rao M, Ye R, Terry-Emert E, Johnson CR, Hanson M, et al. Pollen-mediated gene flow from transgenic to non-transgenic switchgrass (*Panicum virgatum* L.) in the field. *BMC biotechnology*. **17**:40 (2017).

45. Ganie ZA, Jhala AJ. Modeling pollen-mediated gene flow from glyphosate-resistant to-susceptible giant ragweed (*Ambrosia trifida* L.) under field conditions. *Scientific reports*. **7**:17067 (2017).

46. Jansen RC, Den Nijs APM. A statistical mixture model for estimating the proportion of unreduced pollen grains in perennial ryegrass (*Lolium perenne* L.) via the size of pollen grains. *Euphytica*. **70**:205–215 (1993).

47. Griffiths DJ, Pegler RAD, Tonguthaisri T. Cross compatibility between diploid and tetraploid perennial ryegrass (*Lolium perenne* L.). *Euphytica*. **20**:102–12 (1971).

48. Hanzlik K, Gerowitt B. Methods to conduct and analyze weed surveys in arable farming: a review. *Agron Sustain Dev*. **36**:11 (2016).

49. Leeson J, Sheard J, Thomas A. Weed communities associated with arable Saskatchewan farm management systems. *Canadian Journal of Plant Science*. **80**:177–85 (2000).

50. Fried G, Norton LR, Reboud X. Environmental and management factors determining weed species composition and diversity in France. *Agriculture, ecosystems & environment*. **128**:68–76 (2008).

51. Ohadi S, Littlejohn M, Mesgaran M, Rooney W, Bagavathiannan M. Surveying the spatial distribution of feral sorghum (*Sorghum bicolor* L.) and its sympatry with johnsongrass (*S. halepense*) in South Texas. *PLOS ONE*. **13**:e0195511 (2018).

52. Owen MJ, Powles SB. Distribution and frequency of herbicide-resistant wild oat (*Avena* spp.) across the Western Australian grain belt. *Crop Pasture Sci*. **60**:25–31 (2009).

53. Fried G, Chauvel B, Reboud X. A functional analysis of large-scale temporal shifts from 1970 to 2000 in weed assemblages of sunflower crops in France. *Journal of Vegetation Science*. **20**:49–58 (2009).

54. Lutman P, Storkey J, Martin H, Holland J. Abundance of weeds in arable fields in southern England in 2007/08. *Asp Appl Biol*. **91**:163–8 (2009).

55. Leeson J, Sheard J, Thomas A. Multivariate classification of farming systems for use in integrated pest management studies. *Canadian journal of plant science*. **79**:647–54 (1999).

56. Hanson BD, Shrestha A, Shaner DL. Distribution of glyphosate-resistant horseweed (*Conyza canadensis*) and relationship to cropping systems in the Central Valley of California. *Weed Science*. **57**:48–53 (2009).

57. Jasieniuk M, Ahmad R, Sherwood AM, Firestone JL, Perez-Jones A, Lanini WT, et al. Glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in California: distribution, response to glyphosate, and molecular evidence for an altered target enzyme. *Weed Science*. **56**:496–502 (2008).

58. Busi R, Goggin DE, Heap IM, Horak MJ, Jugulam M, Masters RA, et al. Weed resistance to synthetic auxin herbicides. *Pest Management Science*. **74**:2265–76 (2018).

59. Peterson MA, McMaster SA, Riechers DE, Skelton J, Stahlman PW. 2,4-D past, present, and future: a review. *Weed Technology*. **30**:303–45 (2016).

60. Masters RA, Burch PL, Brueninger J, Carrithers VF, Jachetta J, Kline WN, et al. Aminopyralid: a new herbicide for pasture vegetation management. In: Proceedings of the American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America International Annual Meeting; 2005 Nov 10. Salt Lake City, Utah; (2005).

61. Epp JB, Alexander AL, Balko TW, Buysse AM, Brewster WK, Bryan K, et al. The discovery of Arylex™ active and Rinskor™ active: Two novel auxin herbicides. *Bioorganic & Medicinal Chemistry*. **24**:362–71 (2016).

62. Epp JB, Schmitzer PR, Crouse GD. Fifty years of herbicide research: comparing the discovery of trifluralin and halauxifen-methyl. *Pest Management Science*. **74**:9–16 (2018).

63. Christoffoleti PJ, Figueiredo MRA de, Peres LEP, Nissen S, Gaines T, Christoffoleti PJ, et al. Auxinic herbicides, mechanisms of action, and weed resistance: A look into recent plant science advances. *Scientia Agricola*. **72**:356–62 (2015).

64. Devine M, Duke SO, Fedtke C. Physiology of herbicide action. 1st ed. Englewood Cliffs, New Jersey: PTR Prentice Hall; pp. 441 (1992).

65. Fahad S, Hussain S, Matloob A, Khan FA, Khaliq A, Saud S, et al. Phytohormones and plant responses to salinity stress: a review. *Plant Growth Regulation*. **75**:391–404 (2015).

66. Wani SH, Kumar V, Shriram V, Sah SK. Phytohormones and their metabolic engineering for abiotic stress tolerance in crop plants. *The Crop Journal*. **4**:162–76 (2016).

67. Flasiński M, Hąc-Wydro K. Natural vs synthetic auxin: Studies on the interactions between plant hormones and biological membrane lipids. *Environmental Research*. **133**:123–34 (2014).

68. Salehin M, Bagchi R, Estelle M. SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *The Plant Cell*. **27**:9–19 (2015).

69. Rodríguez-Serrano M, Pazmiño DM, Sparkes I, Rochetti A, Hawes C, Romero-Puertas MC, et al. 2,4-Dichlorophenoxyacetic acid promotes S-nitrosylation and oxidation of actin affecting cytoskeleton and peroxisomal dynamics. *J Exp Bot*. **65**:4783–93 (2014).

70. Badescu GO, Napier RM. Receptors for auxin: will it all end in TIRs? *Trends in Plant Science*. **11**:217–23 (2006).

71. Tan X, Calderon-Villalobos LIA, Sharon M, Zheng C, Robinson CV, Estelle M, et al. Mechanism of auxin perception by the TIR1 ubiquitin ligase. *Nature*. **446**:640–5 (2007).

72. Grossmann K. Auxin herbicides: current status of mechanism and mode of action. *Pest Management Science*. **66**:113–20 (2010).

73. Grossmann K. Mediation of herbicide effects by hormone interactions. *Journal of Plant Growth Regulation*. **22**:109–22 (2003).

74. Gao Y, Li J, Pan X, Liu D, Napier R, Dong L. Quinclorac resistance induced by the suppression of the expression of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase genes in *Echinochloa crus-galli* var. *zelayensis*. *Pesticide Biochemistry and Physiology*. **146**:25–32 (2018).

75. Andersson L. Effects of MCPA and tribenuron-methyl on seed production and seed size of annual weeds. *Swedish Journal of Agricultural Research*. **24**:49–56 (1994).

76. Ball DA. Effects of aminocyclopyrachlor herbicide on downy brome (*Bromus tectorum*) seed production under field conditions. *Invasive Plant Science and Management*. **7**:561–4 (2014).

77. Crone EE, Marler M, Pearson DE. Non-target effects of broadleaf herbicide on a native perennial forb: a demographic framework for assessing and minimizing impacts. *Journal of Applied Ecology*. **46**:673–82 (2009).

78. Rinella MJ, Masters RA, Bellows SE. Growth regulator herbicides prevent invasive annual grass seed production under field conditions. *Rangeland ecology & management*. **63**:487–490 (2010).

79. Rinella MJ, Haferkamp MR, Masters RA, Muscha JM, Bellows SE, Vermeire LT. Growth regulator herbicides prevent invasive annual grass seed production. *Invasive Plant Science and Management*. **3**:12–6 (2010).

80. Rinella MJ, Masters RA, Bellows SE. Effects of growth regulator herbicide on downy brome (*Bromus tectorum*) seed production. *Invasive Plant Science and Management*. **6**:60–4 (2013).

81. Schruff MC, Spielman M, Tiwari S, Adams S, Fenby N, Scott RJ. The AUXIN RESPONSE FACTOR 2 gene of *Arabidopsis* links auxin signalling, cell division, and the size of seeds and other organs. *Development*. **133**:251–61 (2006).

82. Zahid HJ, Robinson E, Kelly RL. Agriculture, population growth, and statistical analysis of the radiocarbon record. *PNAS*. **113**:931–5 (2016).

83. Busi R, Gaines TA, Walsh MJ, Powles SB. Understanding the potential for resistance evolution to the new herbicide pyroxasulfone: field selection at high doses versus recurrent selection at low doses. *Weed Research*. **52**:489–499 (2012).

84. Martins BA, Sánchez-Olguín E, Perez-Jones A, Hulting AG, Mallory-Smith C. Alleles contributing to ACCase-resistance in an Italian ryegrass (Lolium perenne ssp. multiflorum) population from Oregon. *Weed science*. **62**:468–473 (2014).

85. Culpepper AS, Whitaker JR, MacRae AW, York AC. Distribution of glyphosate-resistant palmer amaranth (*Amaranthus palmeri*) in Georgia and North Carolina during 2005 and 2006. *J Cotton Sci*. **12**:5 (2008).

86. Hicks HL, Comont D, Coutts SR, Crook L, Hull R, Norris K, et al. The factors driving evolved herbicide resistance at a national scale. *Nature Ecology and Evolution*. **2**:529 (2018).

87. Keshtkar E, Mathiassen SK, Moss SR, Kudsk P. Resistance profile of herbicide-resistant *Alopecurus myosuroides* (black-grass) populations in Denmark. *Crop Protection*. **69**:83–9 (2015).

88. Perez-Jones A, Park KW, Colquhoun J, Mallory-Smith C, Shaner D. Identification of glyphosate-resistant Italian ryegrass (*Lolium multiflorum*) in Oregon. *Weed Science*. **53**:775–779 (2005).

89. Boryan C, Yang Z, Mueller R, Craig M. Monitoring US agriculture: the US Department of Agriculture, National Agricultural Statistics Service, Cropland Data layer program. *Geocarto International*. **26**:341–58 (2011).

90. Ali A, Streibig JC, Duus J, Andreasen C. Use of image analysis to assess color response on plants caused by herbicide application. *Weed Technology*. **27**:604–11 (2013).

91. Ridler T, Calvard S. Picture thresholding using an iterative selection method. *IEEE trans syst Man Cybern*. **8**:630–2 (1978).

92. Tomasi C, Manduchi R. Bilateral filtering for gray and color images. In: Proceedings of theIEEE International Conference on Computer Vision; 1998 Jan 4; Bombay, India; (1998).

93. Beckie HJ, Heap IM, Smeda RJ, Hall LM. Screening for herbicide resistance in weeds. *Weed Technology*. **14**:428–45 (2000).

94. Murray BG, Friesen LF, Beaulieu KJ, Morrison IN. A seed bioassay to Identify Acetyl-CoA carboxylase inhibitor resistant wild oat (*Avena fatua*) populations. *Weed Technology*. **10**:85–9 (1996).

95. Tal A, Kotoula-Syka E, Rubin B. Seed-bioassay to detect grass weeds resistant to acetyl coenzyme A carboxylase inhibiting herbicides. *Crop Protection*. **19**:467–72 (2000).

96. Greilhuber J, Doležel J, Lysák MA, Bennett MD. The origin, evolution and proposed stabilization of the terms ‘genome size’ and ‘C-value’ to describe nuclear DNA contents. *Annals of Botany*. **95**:255–260 (2005).

97. Contreras RN, Shearer K. Genome size, ploidy, and base composition of wild and cultivated acer. *Journal of the American Society for Horticultural Science*. **143**:470–85 (2018).

98. Doležel J, Greilhuber J, Suda J. Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols*. **2**:2233–44 (2007).

99. Lattier JD, Chen H, Contreras RN. Variation in genome size, ploidy, stomata, and rDNA signals in *Althea*. *Journal of the American Society for Horticultural Science*. **144**:130–140 (2019).

100. Rothleutner JJ, Friddle MW, Contreras RN. Ploidy levels, relative genome sizes, and base pair composition in *Cotoneaster*. *Journal of the American Society for Horticultural Science*. **141**:457–66 (2016).

101. Weihing RM. Registration of Gulf annual ryegrass (Reg. No. 8). *Crop Science*. **3**:366–366 (1963).

102. Nelson LR, Crowder J, Turner FT, Evers GW, Rouquette FM. Registration of 'TAMTBO' annual ryegrass. *Journal of Plant Registrations*. **1**:127–8 (2007).

103. Lumley T. Analysis of complex survey samples. *Journal of Statistical Software*. **9**:1–19 (2004).

104. Rampton HH, Ching TM. Longevity and dormancy in seeds of several cool-season grasses and legumes buried in soil. *Agron`omy Journal*. **58**:220–2 (1966 4/01).

105. Brewster DB, Donaldson SW, Mallory-Smith C. Longevity of dicoflop-resistant Italian ryegrass seed in soil. In: Vol. 48, Proceedings - Western Society of Weed Science Annual meeting; 1995 March 13-15; Sacramento, California; (1995).

106. Buhler DD, Hartzler RG, Forcella F. Implications of weed seedbank dynamics to weed management. *Weed Science*. **45**:329–36 (1997).

107. Davis AS. When does it make sense to target the weed seed bank? *Weed Science*. **54**:558–65 (2006).

108. Kumaratilake AR, Preston C. Low temperature reduces glufosinate activity and translocation in wild radish (*Raphanus raphanistrum*). *Weed science*. **53**:10–16 (2005).

109. Ganie ZA, Jugulam M, Jhala AJ. Temperature influences efficacy, absorption, and translocation of 2,4-D or glyphosate in glyphosate-resistant and glyphosate-susceptible common ragweed (*Ambrosia artemisiifolia*) and giant ragweed (*Ambrosia trifida*). *Weed Science; Lawrence*. **65**:588–602 (2017).

110. Xie HS, Hsiao AI, Quick WA. Influence of temperature and light intensity on absorption, translocation, and phytotoxicity of fenoxaprop-ethyl and imazamethabenz-methyl in *Avena fatua*. *Journal of Plant Growth Regulation*. **15**:57–62 (1996).

111. Bagavathiannan MV, Davis AS. An ecological perspective on managing weeds during the great selection for herbicide resistance. *Pest Manag Sci*. **74**:2277–2286 (2018).

112. Evans JA, Tranel PJ, Hager AG, Schutte B, Wu C, Chatham LA, et al. Managing the evolution of herbicide resistance. *Pest Manag Sci*. **72**:74–80 (2016).

113. Hurley TM, Frisvold G. Economic barriers to herbicide-resistance management. *Weed Science*. **64**:585–594 (2016).

114. Nichiyama I, Inomata N. Embryological studies on cross-incompatibility between 2x and 4x in *Brassica*. *The Japanese Journal of Genetics*. **41**:27–42 (1966).

115. Watkins AE. Hybrid sterility and incompatibility. *Journal of Genetics*. **25**:125–162 (1932).

116. Gill MS, Bajaj YPS. Hybridization between diploid (*Gossypium arboreum*) and tetraploid (*Gossypium hirsutum*) cotton through ovule culture. *Euphytica*. **36**:625–630 (1987).

117. Hay J. Gains to the grower from weed science. *Weed Science*. **22**:439–42 (1974).

118. Steadman KJ, Ellery AJ, Chapman R, Moore A, Turner NC. Maturation temperature and rainfall influence seed dormancy characteristics of annual ryegrass (*Lolium rigidum*). *Australian Journal of Agricultural Research*. **55**:1047–57 (2004).

119. Bagavathiannan MV, Norsworthy JK. Late-season seed production in arable weed communities: management implications. *Weed science*. **60**:325–34 (2012).

120. Young FL, Whitesides RE. Efficacy of postharvest herbicides on Russian thistle (*Salsola iberica*) control and seed germination. *Weed Science*. **35**:554–9 (1987).

121. Cousens R, Mortimer M. Dynamics of weed populations. Cambridge University Press; pp. 332 (1995).

122. Friesen H, Born WV, Keys C, Dryden R, Molberg E, Siemens B. Effect of time of application and dosage of dicamba on the tolerance of wheat, oats and barley. *Canadian Journal of Plant Science*. **48**:213–5 (1968).

123. Rinella MJ, Kells JJ, Ward RW. Response of 'Wakefield'winter wheat (Triticum aestivum) to dicamba. *Weed Technology*. **15**:523–9 (2001).

124. Sikkema PH, Brown L, Shropshire C, Soltani N. Responses of three types of winter wheat (*Triticum aestivum* L.) to spring-applied post-emergence herbicides. *Crop Protection*. **26**:715–20 (2007).

125. Sheley RL, Duncan CA, Halstvedt MB, Jacobs JS. Spotted knapweed and grass response to herbicide treatments. *Journal of Range Management*. **53**:176–82 (2000).

126. Shinn SL, Thill DC. Tolerance of several perennial grasses to imazapic. *Weed Technology*. **18**:60–5 (2004).

127. Kanno Y, Jikumaru Y, Hanada A, Nambara E, Abrams SR, Kamiya Y, et al. Comprehensive hormone profiling in developing *Arabidopsis* seeds: examination of the site of ABA biosynthesis, ABA transport and hormone interactions. *Plant Cell Physiol*. **51**:1988–2001 (2010).

128. Rock CD, Quatrano RS. The role of hormones during deed development. In: Davies PJ, editor. *Plant Hormones: Physiology, Biochemistry and Molecular Biology [Internet]*. Dordrecht, Netherlands: Springer; p.671–97 (1995). Available from: https://doi.org/10.1007/978-94-011-0473-9_31

129. Baalbaki R, Elias SG, Marcos Filho J, McDonald, MB. Seed vigor testing handbook. 32nd ed. Ithaca, New York: Association of official seed analysis; pp. 88 (2009).

130. Elias SG, Copeland LO, McDonald MB, Baalbaki RZ. Seed testing: principles and practices. 1st ed. East Lansing, Michigan: Michigan State University Press; pp. 364 (2012).

131. Marcos Filho J, Marcos Filho J. Seed vigor testing: an overview of the past, present and future perspective. *Scientia Agricola*. **72**:363–74 (2015).

132. Fei S, Nelson E. Estimation of Pollen Viability, Shedding Pattern, and Longevity of Creeping Bentgrass on Artificial Media. *Crop Science*. **43**:2177–81 (2003).

133. Ahloowalia B. Germination in vitro of ryegrass pollen grains. *Euphytica*. **22**:575–81 (1973).

134. Tuinstra M, Wedel J. Estimation of pollen viability in grain sorghum. *Crop Science*. **40**:968–70 (2000).

135. Bolker B. Dealing with quasi-models in R. *Compare*. **1**:5–452305 (2017).

136. Ritz C, Pipper CB, Streibig JC. Analysis of germination data from agricultural experiments. *European Journal of Agronomy*. **45**:1–6 (2013).

137. Venables W, Ripley B. Modern applied statistics with S. 4th ed. New York: Springer; (2002).

138. Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S, et al. Simultaneous inference in general parametric models. *Biometrical Journal*. **50**:346–63 (2016).

139. Knezevic SZ, Streibig JC, Ritz C. Utilizing R software package for dose-response studies: the concept and data analysis. *Weed Technology*. **21**:840–8 (2007).

140. Kyser GB, Peterson VF, Davy JS, DiTomaso JM. Preemergent control of medusahead on California annual rangelands with aminopyralid. *Rangeland Ecology & Management*. **65**:418–25 (2012).

141. Lewis DF, Roten RL, Everman WJ, Gannon TW, Richardson RJ, Yelverton FH. Absorption, translocation, and metabolism of aminocyclopyrachlor in tall fescue (*Lolium arundinaceum*). *Weed science*. **61**:348–52 (2013).

142. Strachan SD, Casini MS, Heldreth KM, Scocas JA, Nissen SJ, Bukun B, et al. Vapor movement of synthetic auxin herbicides: aminocyclopyrachlor, aminocyclopyrachlor-methyl ester, dicamba, and aminopyralid. *Weed science*. **58**:103–108 (2010).

143. Strachan SD, Ferry NM, Cooper TL. Vapor movement of aminocyclopyrachlor, aminopyralid, and dicamba in the field. *Weed technology*. **27**:143–155 (2013).

144. Fast BJ, Ferrell JA, MacDonald GE, Krutz LJ, Kline WN. Picloram and aminopyralid sorption to soil and clay minerals. *Weed science*. **58**:484–9 (2010).

145. Walsh TA, Neal R, Merlo AO, Honma M, Hicks GR, Wolff K, et al. Mutations in an Auxin Receptor Homolog AFB5 and in SGT1b Confer Resistance to Synthetic Picolinate Auxins and Not to 2,4-Dichlorophenoxyacetic Acid or Indole-3-Acetic Acid in *Arabidopsis*. *Plant Physiology*. **142**:542–52 (2006).

# APPENDIX A

Table A-1 Surveyed field information and coordinates

| ID | Latitude | Longitude | Elevation | Crop | Date of collection | Ryegrass presence | Density* | Ploidy |
|---|---|---|---|---|---|---|---|---|
| 001 | 45° 33' 48"N | 123° 07' 10"W | 62.130 | Clover | 2018.06.25 | absent | Zero | - |
| 002 | 45° 33' 13"N | 123° 06' 56"W | 56.100 | Orchardgrass | 2016.07.08 | present | Medium | Diploid |
| 003 | 45° 33' 00"N | 123° 07' 41"W | 62.611 | Tall fescue | 2018.06.25 | absent | Zero | - |
| 004 | 45° 32' 26"N | 123° 06' 53"W | 57.324 | Tall fescue | 2018.06.25 | Not present | Zero | - |
| 005 | 45° 30' 20"N | 123° 00' 42"W | 52.517 | Wheat | 2018.06.25 | present | High | diploid |
| 006 | 45° 29' 19"N | 123° 07' 12"W | 63.813 | Tall fescue | 2018.06.25 | absent | Zero | - |
| 007 | 45° 28' 59"N | 123° 03' 20"W | 58.200 | Wheat | 2016.06.16 | present | High | diploid |
| 008 | 45° 28' 31"N | 122° 59' 10"W | 58.526 | Wheat | 2018.06.25 | present | Medium | diploid |
| 009 | 45° 28' 22"N | 123° 02' 12"W | 58.766 | Tall fescue | 2018.06.25 | absent | Zero | - |
| 010 | 45° 28' 21"N | 123° 01' 04"W | 62.611 | Wheat | 2018.06.25 | absent | Zero | - |
| 011 | 45° 26' 31"N | 122° 55' 37"W | 56.363 | Tree crop | 2018.06.25 | absent | Zero | - |
| 012 | 45° 25' 58"N | 122° 52' 40"W | 90.249 | Tall fescue | 2018.06.25 | present | Medium | diploid |
| 013 | 45° 25' 58"N | 122° 52' 40"W | 90.249 | Wheat | 2018.06.25 | absent | Zero | - |
| 014 | 45° 25' 58"N | 122° 52' 40"W | 90.249 | Wheat | 2018.06.25 | absent | Zero | - |
| 015 | 45° 25' 58"N | 123° 06' 12"W | 104.428 | Wheat | 2018.06.25 | present | Low | diploid |
| 016 | 45° 25' 06"N | 122° 58' 35"W | 65.976 | Wheat | 2018.06.25 | present | Low | diploid |
| 017 | 45° 23' 58"N | 123° 06' 02"W | 64.774 | Tall fescue | 2018.06.25 | present | High | diploid |
| 018 | 45° 23' 50"N | 123° 06' 39"W | 61.169 | Wheat | 2018.07.03 | present | High | diploid |
| 019 | 45° 22' 59"N | 122° 53' 18"W | 108.754 | Wheat | 2018.06.25 | present | High | diploid |
| 020 | 45° 20' 31"N | 123° 12' 06"W | 59.967 | Tall fescue | 2018.07.03 | present | Low | diploid |
| 021 | 45° 15' 08"N | 123° 08' 23"W | 46.990 | Tall fescue | 2018.07.03 | absent | Zero | - |
| 022 | 45° 15' 07"N | 123° 10' 48"W | 46.990 | Tall fescue | 2018.07.03 | present | Low | diploid |
| 023 | 45° 14' 29"N | 122° 57' 07"W | 54.600 | Tall fescue | 2017.07.17 | present | Low | diploid |

| Continued | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 024 | 45° 13' 27"N | 122° 51' 38"W | 55.300 | Orchardgrass | 2017.05.16 | present | Low | diploid |
| 025 | 45° 13' 18"N | 123° 09' 10"W | 45.788 | Tall fescue | 2018.07.03 | present | Medium | diploid |
| 026 | 45° 13' 06"N | 122° 59' 35"W | 52.300 | Orchardgrass | 2017.05.20 | present | High | diploid |
| 027 | 45° 12' 37"N | 123° 06' 16"W | 49.633 | Tall fescue | 2018.07.03 | absent | Zero | - |
| 028 | 45° 12' 34"N | 122° 53' 27"W | 47.951 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 029 | 45° 11' 28"N | 123° 06' 54"W | 49.633 | Tall fescue | 2018.07.03 | absent | Zero | - |
| 030 | 45° 10' 35"N | 122° 54' 48"W | 52.998 | Wheat | 2018.06.26 | present | Medium | diploid |
| 031 | 45° 10' 35"N | 122° 38' 25"W | 80.100 | Tree crop | 2017.05.01 | present | High | diploid |
| 032 | 45° 10' 07"N | 122° 48' 50"W | 62.851 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 033 | 45° 07' 29"N | 123° 11' 03"W | 141.000 | Wheat | 2017.07.17 | present | High | diploid |
| 034 | 45° 06' 30"N | 123° 18' 16"W | 46.700 | Orchardgrass | 2017.07.17 | absent | Zero | - |
| 035 | 45° 06' 27"N | 123° 11' 26"W | 51.400 | Tall fescue | 2017.07.17 | absent | Zero | - |
| 036 | 45° 06' 18"N | 123° 20' 41"W | 53.300 | Tall fescue | 2017.07.17 | present | Medium | diploid |
| 037 | 45° 06' 04"N | 123° 17' 57"W | 62.400 | Tall fescue | 2017.07.17 | absent | Zero | - |
| 038 | 45° 04' 48"N | 123° 17' 60"W | 47.900 | Tall fescue | 2017.07.17 | absent | Zero | - |
| 039 | 45° 04' 46"N | 123° 15' 58"W | 52.100 | Tree crop | 2017.07.17 | present | High | diploid |
| 040 | 45° 04' 01"N | 123° 18' 14"W | 55.400 | Tall fescue | 2017.07.17 | present | Medium | diploid |
| 041 | 45° 03' 41"N | 122° 48' 33"W | 44.200 | Tall fescue | 2017.07.20 | Not present | Zero | - |
| 042 | 45° 03' 33"N | 123° 17' 57"W | 55.700 | Tall fescue | 2017.07.17 | present | Low | diploid |
| 043 | 45° 03' 10"N | 122° 44' 28"W | 85.700 | Tall fescue | 2017.06.22 | present | High | diploid |
| 044 | 45° 02' 55"N | 122° 54' 40"W | 37.864 | Wheat | 2018.07.16 | present | High | diploid |
| 045 | 45° 02' 50"N | 122° 48' 38"W | 51.796 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 046 | 45° 01' 54"N | 123° 20' 09"W | 59.500 | Tall fescue | 2017.06.27 | present | Low | diploid |
| 047 | 45°01' 28"N | 123° 24' 10"W | 151.800 | Tall fescue | 2017.07.17 | absent | Zero | - |
| 048 | 45° 00' 22"N | 122° 51' 06"W | 60.800 | Wheat | 2017.07.20 | present | High | diploid |
| 049 | 44° 59' 43"N | 122° 55' 14"W | 59.000 | Tall fescue | 2017.07.20 | absent | Zero | - |
| 050 | 44° 59' 29"N | 122° 53' 21"W | 56.603 | Tall fescue | 2018.06.26 | present | Low | tetraploid |
| 051 | 44° 58' 13"N | 122° 53' 10"W | 67.177 | Tall fescue | 2018.06.26 | absent | Zero | - |

| Continued | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 052 | 44° 57' 21"N | 122° 53' 02"W | 69.800 | Orchardgrass | 2016.04.02 | present | High | diploid |
| 053 | 44° 56' 13"N | 122° 53' 05"W | 79.434 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 054 | 44° 55' 26"N | 122° 42' 26"W | 364.900 | Wheat | 2017.07.20 | present | Medium | diploid |
| 055 | 44° 55' 22"N | 122° 44' 08"W | 275.000 | Tall fescue | 2017.07.20 | present | Low | diploid |
| 056 | 44° 54' 41"N | 122° 45' 13"W | 267.900 | Wheat | 2017.06.22 | present | High | diploid |
| 057 | 44° 54' 13"N | 122° 42' 07"W | 350.600 | Wheat | 2017.07.20 | present | High | diploid |
| 058 | 44° 53' 41"N | 122° 41' 45"W | 368.800 | Tall fescue | 2017.07.20 | absent | Zero | - |
| 059 | 44° 53' 27"N | 122° 46' 58"W | 224.300 | Tall fescue | 2017.07.20 | absent | Zero | - |
| 060 | 44° 53' 40"N | 123° 15' 25"W | 81.900 | Tree crop | 2017.08.11 | present | High | diploid |
| 061 | 44° 52' 39"N | 122° 50' 35"W | 161.626 | Wheat | 2018.06.26 | absent | Zero | - |
| 062 | 44° 52' 36"N | 122° 47' 38"W | 191.900 | Tall fescue | 2017.07.20 | absent | Zero | - |
| 063 | 44° 52' 15"N | 122° 45' 18"W | 240.694 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 064 | 44° 52' 90"N | 123° 15' 40"W | 93.854 | Oat | 2018.06.29 | absent | Zero | - |
| 065 | 44° 51' 80"N | 123° 15' 41"W | 81.400 | Tall fescue | 2017.07.12 | present | High | diploid |
| 066 | 44° 50' 54"N | 122° 44' 08"W | 244.780 | Wheat | 2018.06.26 | absent | Zero | - |
| 067 | 44° 50' 20"N | 123° 08' 18"W | 46.600 | Tall fescue | 2017.07.20 | present | Low | diploid |
| 068 | 44° 49' 22"N | 123° 15' 05"W | 76.800 | Orchardgrass | 2017.07.12 | present | Medium | diploid |
| 069 | 44° 48' 45"N | 122° 42' 55"W | 286.116 | Tall fescue | 2018.06.26 | present | Low | diploid |
| 070 | 44° 48' 45"N | 122° 56' 53"W | 96.017 | Tall fescue | 2018.06.26 | present | High | diploid |
| 071 | 44° 48' 42"N | 123° 13' 40"W | 87.800 | Tall fescue | 2017.07.12 | absent | Zero | - |
| 072 | 44° 48' 40"N | 122° 38' 58"W | 347.640 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 073 | 44° 48' 12"N | 123° 13' 33"W | 77.900 | Tall fescue | 2017.07.12 | present | High | diploid |
| 074 | 44° 48' 10"N | 122° 57' 37"W | 100.102 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 075 | 44° 48' 06"N | 122° 54' 48"W | 105.149 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 076 | 44° 48' 05"N | 123° 09' 35"W | 52.037 | Tall fescue | 2018.06.29 | present | Low | diploid |
| 077 | 44° 48' 02"N | 123° 19' 47"W | 73.200 | Orchardgrass | 2017.07.12 | absent | Zero | - |
| 078 | 44° 47' 32"N | 123° 09' 05"W | 53.238 | Tall fescue | 2018.06.29 | absent | Zero | - |
| 079 | 44° 47' 27"N | 123° 19' 47"W | 69.100 | Oat | 2017.07.12 | present | High | diploid |

| Continued | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 080 | 44° 46' 60"N | 123° 02' 58"W | 72.500 | Orchardgrass | 2017.07.20 | present | Low | diploid |
| 081 | 44° 46' 50"N | 123° 21' 38"W | 74.100 | Tall fescue | 2017.07.12 | absent | Zero | - |
| 082 | 44° 46' 26"N | 123° 15' 46"W | 72.945 | Tall fescue | 2018.06.29 | present | Low | diploid |
| 083 | 44° 46' 10"N | 123° 01' 03"W | 75.348 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 084 | 44° 46' 06"N | 123° 00' 42"W | 72.464 | Clover | 2018.06.26 | absent | Zero | - |
| 085 | 44° 45' 19"N | 123° 18' 52"W | 87.125 | Tall fescue | 2018.06.29 | absent | Zero | - |
| 086 | 44° 45' 01"N | 123° 15' 55"W | 100.800 | Tall fescue | 2017.07.06 | present | Medium | diploid |
| 087 | 44° 45' 01"N | 123° 15' 55"W | 100.800 | Tall fescue | 2017.07.06 | present | Low | diploid |
| 088 | 44° 44' 39"N | 123° 14' 48"W | 81.100 | Tall fescue | 2017.07.12 | absent | Zero | - |
| 089 | 44° 44' 10"N | 122° 56' 34"W | 92.412 | Wheat | 2018.06.26 | absent | Zero | - |
| 090 | 44° 43' 57"N | 123° 01' 13"W | 72.945 | Tall fescue | 2018.06.26 | absent | Zero | - |
| 091 | 44° 43' 37"N | 122° 50' 30"W | 133.600 | Wheat | 2016.07.08 | present | Low | diploid |
| 092 | 44° 43' 32"N | 123° 00' 03"W | 96.200 | Wheat | 2016.05.12 | present | Medium | diploid |
| 093 | 44° 43' 13"N | 122° 37' 31"W | 222.669 | Tall fescue | 2018.06.20 | absent | Zero | - |
| 094 | 44° 42' 56"N | 122° 50' 26"W | 100.400 | Tree crop | 2016.06.29 | present | Medium | diploid |
| 095 | 44° 42' 40"N | 123° 03' 40"W | 63.332 | Tall fescue | 2018.06.20 | present | Low | diploid |
| 096 | 44° 42' 36"N | 123° 14' 30"W | 72.705 | Tall fescue | 2018.06.20 | present | High | diploid |
| 097 | 44° 42' 30"N | 122° 59' 27"W | 73.906 | Orchardgrass | 2018.06.20 | present | Low | diploid |
| 098 | 44° 42' 28"N | 122° 55' 36"W | 91.210 | Tall fescue | 2018.06.20 | absent | Zero | - |
| 099 | 44° 38' 50"N | 122° 47' 14"W | 110.436 | Tall fescue | 2018.06.20 | present | Low | tetraploid |
| 100 | 44° 35' 13"N | 122° 55' 31"W | 92.892 | Tall fescue | 2018.06.20 | absent | Zero | - |
| 101 | 44° 33' 59"N | 123° 17' 07"W | 119.809 | Wheat | 2018.06.20 | absent | Zero | - |
| 102 | 44° 33' 25"N | 123° 13' 46"W | 65.255 | Tall fescue | 2018.06.18 | absent | Zero | - |
| 103 | 44° 32' 13"N | 123° 18' 47"W | 81.000 | Tall fescue | 2017.07.06 | present | Low | diploid |
| 104 | 44° 31' 30"N | 123° 12' 22"W | 69.581 | Tall fescue | 2018.06.18 | absent | Zero | - |
| 105 | 44° 31' 15"N | 123° 20' 15"W | 74.868 | Tall fescue | 2018.06.21 | present | High | diploid |
| 106 | 44° 31' 11"N | 123° 14' 31"W | 69.821 | Tall fescue | 2018.06.19 | absent | Zero | - |
| 107 | 44° 30' 52"N | 123° 16' 11"W | 73.800 | Tall fescue | 2017.07.10 | absent | Zero | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Continued** | | | | | | | | |
| 108 | 44° 30' 17"N | 123° 03' 01"W | 84.481 | Tall fescue | 2018.06.20 | present | Low | diploid |
| 109 | 44° 29' 52"N | 123° 18' 56"W | 71.744 | Tall fescue | 2018.06.21 | absent | Zero | - |
| 110 | 44° 29' 28"N | 123° 12' 41"W | 74.387 | Tall fescue | 2018.06.18 | absent | Zero | - |
| 111 | 44° 28' 35"N | 123° 18' 47"W | 75.600 | Tall fescue | 2017.07.06 | absent | Zero | - |
| 112 | 44° 27' 43"N | 123° 14' 90"W | 74.387 | Wheat | 2018.06.19 | present | Low | diploid |
| 113 | 44° 27' 33"N | 123° 12' 40"W | 77.271 | Tall fescue | 2018.06.18 | absent | Zero | - |
| 114 | 44° 27' 24"N | 123° 17' 43"W | 76.069 | Tall fescue | 2018.06.21 | absent | Zero | - |
| 115 | 44° 27' 04"N | 123° 14' 20"W | 74.400 | Tall fescue | 2017.07.06 | present | Low | diploid |
| 116 | 44° 26' 53"N | 123° 16' 29"W | 78.100 | Tall fescue | 2017.07.06 | present | High | tetraploid |
| 117 | 44° 26' 52"N | 123° 16' 43"W | 78.000 | Tall fescue | 2017.07.06 | absent | Zero | - |
| 118 | 44° 26' 42"N | 123° 15' 08"W | 78.713 | Tall fescue | 2018.06.21 | present | Low | tetraploid |
| 119 | 44° 26' 41"N | 123° 19' 06"W | 75.900 | Tall fescue | 2017.07.06 | absent | Zero | - |
| 120 | 44° 25' 47"N | 123° 02' 09"W | 60.027 | Tree crop | 2018.07.17 | present | High | diploid |
| 121 | 44° 25' 31"N | 123° 06' 19"W | 83.039 | Clover | 2018.06.20 | absent | Zero | - |
| 122 | 44° 25' 22"N | 123° 14' 04"W | 75.100 | Tall fescue | 2017.07.06 | absent | Zero | - |
| 123 | 44° 25' 10"N | 123° 16' 27"W | 78.000 | Tall fescue | 2017.07.06 | present | High | diploid |
| 124 | 44° 24' 30"N | 123° 08' 24"W | 82.700 | Wheat | 2017.07.10 | present | Low | tetraploid |
| 125 | 44° 24' 28"N | 123° 12' 14"W | 81.116 | Tall fescue | 2018.06.18 | absent | Zero | - |
| 126 | 44° 24' 08"N | 123° 07' 27"W | 84.400 | Tall fescue | 2017.07.10 | present | High | diploid |
| 127 | 44° 23' 52"N | 123° 16' 56"W | 80.400 | Tall fescue | 2017.07.06 | present | Medium | diploid |
| 128 | 44° 23' 35"N | 123° 09' 28"W | 82.078 | Tall fescue | 2018.06.18 | absent | Zero | - |
| 129 | 44° 23' 21"N | 123° 08' 09"W | 85.442 | Tall fescue | 2018.06.18 | absent | Zero | - |
| 130 | 44° 23' 16"N | 122° 57' 26"W | 104.668 | Orchardgrass | 2018.06.20 | present | Medium | diploid |
| 131 | 44° 22' 46"N | 123° 21' 19"W | 114.100 | Tall fescue | 2017.07.10 | present | High | diploid |
| 132 | 44° 22' 44"N | 123° 06' 57"W | 87.000 | Tall fescue | 2017.07.10 | absent | Zero | - |
| 133 | 44° 21' 41"N | 123° 20' 07"W | 95.800 | Tall fescue | 2017.07.10 | present | High | diploid |
| 134 | 44° 21' 38"N | 123° 18' 20"W | 83.400 | Tall fescue | 2017.07.10 | absent | Zero | - |

| Continued | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 135 | 44° 21' 34"N | 123° 03' 10"W | 92.892 | Clover | 2018.06.18 | present | High | diploid |
| 136 | 44° 21' 09"N | 123° 05' 15"W | 91.450 | Tall fescue | 2018.06.20 | absent | Zero | - |
| 137 | 44° 21' 01"N | 123° 08' 21"W | 86.400 | Tall fescue | 2017.07.10 | present | High | diploid |
| 138 | 44° 20' 55"N | 123° 05' 16"W | 119.809 | Tall fescue | 2018.06.19 | absent | Zero | - |
| 139 | 44° 19' 39"N | 123° 18' 03"W | 94.900 | Tall fescue | 2017.07.10 | absent | Zero | - |
| 140 | 44° 18' 59"N | 123° 15' 07"W | 81.116 | Wheat | 2018.06.19 | absent | Zero | - |
| 141 | 44° 18' 59"N | 123° 16' 41"W | 75.829 | Orchardgrass | 2018.06.21 | absent | Zero | - |
| 142 | 44° 18' 06"N | 123° 14' 25"W | 73.906 | Tall fescue | 2018.06.19 | absent | Zero | - |
| 143 | 44° 16' 48"N | 123° 03' 22"W | 98.100 | Tall fescue | 2017.07.11 | present | Medium | diploid |
| 144 | 44° 16' 48"N | 123° 08' 14"W | 94.100 | Orchardgrass | 2016.07.08 | present | High | diploid |
| 145 | 44° 13' 10"N | 123° 08' 33"W | 100.600 | Tall fescue | 2017.07.10 | absent | Zero | - |
| 146 | 44° 12' 22"N | 123° 04' 47"W | 108.800 | Tall fescue | 2017.07.11 | present | Medium | diploid |
| 147 | 44° 09' 15"N | 123° 11' 05"W | 108.700 | Orchardgrass | 2017.07.16 | present | High | diploid |
| 148 | 44° 02' 11"N | 123° 16' 26"W | 88.155 | Tall fescue | 2018.07.18 | absent | Zero | - |
| 149 | 44° 00' 19"N | 122° 51' 53"W | 310.400 | Tall fescue | 2017.07.20 | present | Low | diploid |
| 150 | 43° 58' 45"N | 123° 00' 19"W | 106.549 | Tall fescue | 2018.07.19 | absent | Zero | - |

*Low: less than 9 plants/$m^2$; Medium: 9-19 plants/ $m^2$; High: 20 or more plants/$m^2$

Table A-2. Dose response rates used for definition of screening rate for pre-emergent test

| | Flufenacet + metribuzin | Pyroxasulfone | Pronamide |
|---|---|---|---|
| **Treatment** | **Concentration (uM)** | | |
| 1 | 0.0000 | 0.0000 | 0.0000 |
| 2 | 0.0006 | 0.0010 | 0.0010 |
| 3 | 0.0060 | 0.0100 | 0.0100 |
| 4 | 0.0180 | 0.1000 | 0.1000 |
| 5 | 0.0600 | 0.5000 | 1.0000 |
| 6 | 0.6000 | 1.0000 | 5.0000 |
| 7 | 1.8000 | 10.0000 | 10.0000 |
| 8 | 6.0000 | 50.0000 | 20.0000 |
| 9 | 60.0000 | 100.0000 | 50.0000 |

Table A-3 Four-parameters for log-logistic dose response for each herbicide and populations tested.

| **Flufenacet + Metribuzin** | | | | |
|---|---|---|---|---|
| **Population** | **Slope (SE)** | **Upper (SE)** | **Lower (SE)** | **ED50 (SE)** |
| **Gulf** | 0.78 (±0.11) | 128.53(±4.58) | 11.43 (±2.12) | 0.10(±0.02) |
| **DK** | 0.26 (±0.03) | 129.66(±6.43) | 9.45 (±1.58) | 0.72(±0.42) |
| **DAN** | 0.63 (±0.08) | 149.7(±5.28) | 10.24 (±1.25) | 0.08(±0.02) |
| **PR** | 0.35 (±0.06) | 103.3(±5.01) | 8.24 (±3.24) | 3.84(±1.89) |
| **Pyroxasulfone** | | | | |
| **Population** | **Slope (SE)** | **Upper (SE)** | **Lower (SE)** | **ED50 (SE)** |
| **Gulf** | 0.20(±0.01) | 49.61(±2.53) | 11.43 (±2.12) | $4.12x10^{-4}$($\pm1.91x10^{-4}$) |
| **Tetraploid** | 0.22(±0.01) | 65.65(±3.36) | 9.45 (±1.58) | $5.92x10^{-4}$($\pm2.92x10^{-4}$) |
| **Pronamide** | | | | |
| **Population** | **Slope (SE)** | **Upper (SE)** | **Lower (SE)** | **ED50 (SE)** |
| **Gulf** | 5.24(±2.37) | 48.54(±1.81) | 8.88 (±0.63) | 0.49(±2.56) |
| **Tetraploid** | 4.59(±1.43) | 67.07(±2.53) | 12.35 (±0.69) | 0.19(±0.11) |

Table A-4 Frequency of presence of Italian ryegrass on surveyed fields per county.

| | Not present | Present | Total | County | Total |
|---|---|---|---|---|---|
| **Stratum** | **Number of fields** | | | **Frequency (%)** | |
| Benton | 27 | 21 | 48 | 44 | 14 |
| Clackamas | 0 | 1 | 1 | 100 | 1 |
| Linn | 4 | 8 | 12 | 67 | 5 |
| Marion | 20 | 18 | 38 | 47 | 12 |
| Polk | 7 | 8 | 15 | 53 | 5 |
| Washington | 9 | 9 | 18 | 50 | 6 |
| Yamhill | 8 | 10 | 18 | 56 | 7 |
| **Total** | **75** | **75** | **150** | **50** | **50** |

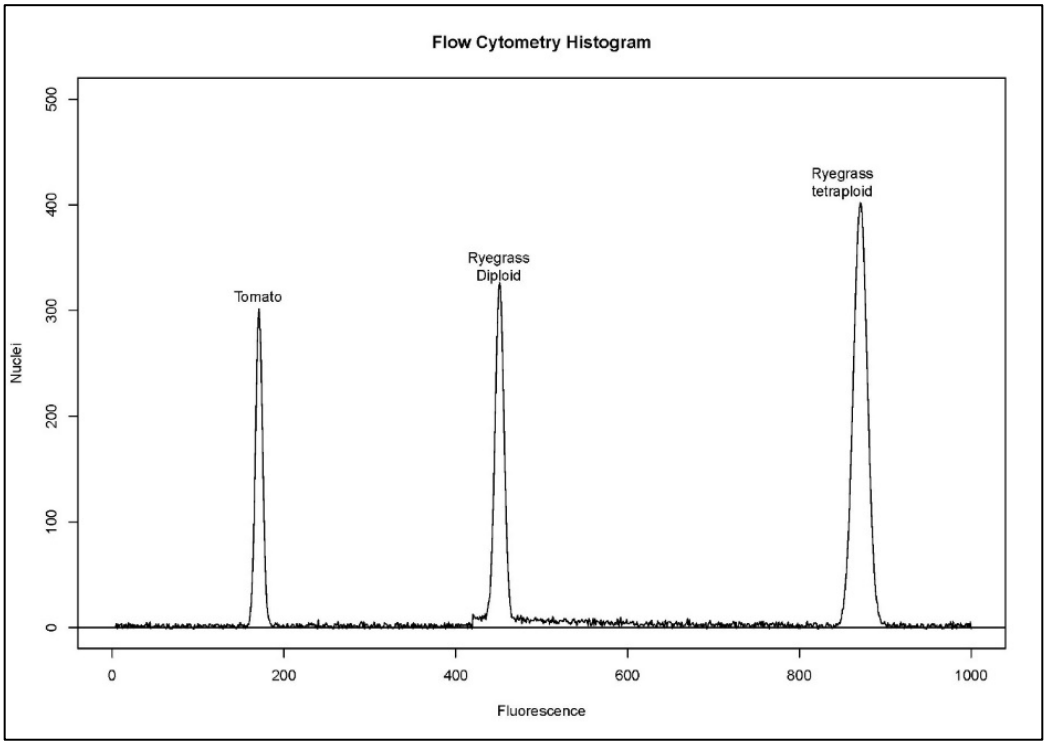



Figure A-1 Flow cytometry histogram showing DNA peaks from standards used (diploid tomato, tetraploid and diploid cultivars of Italian Ryegrass)

Table A-5. Frequency of each density level according to the crop present.

| | Italian ryegrass density (plants/m$^2$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | High† | | | Medium† | | | Low† | | |
| Crop | Fields | Total | Frequency (%) | Fields | Total | Frequency (%) | Fields | Total | Frequency (%) |
| Oat | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Orchard grass | 4 | 10 | 40 | 3 | 10 | 30 | 3 | 10 | 30 |
| Tall fescue | 13 | 40 | 33 | 8 | 40 | 20 | 19 | 40 | 48 |
| Tree crop | 4 | 5 | 80 | 1 | 5 | 20 | 0 | 5 | 0 |
| Wheat | 9 | 18 | 50 | 4 | 18 | 22 | 5 | 18 | 28 |
| White Clover | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |

†: high (20≥ plants/m$^2$), medium (10 to 19 plants/m$^2$) and low (1 to 9 plants/m$^2$).

Table A-6 Frequency of Italian ryegrass density per county on the surveyed area.

| | Density of Italian ryegrass (plants/m$^2$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | High† | | | Medium† | | | Low† | | |
| Crop | Field | Total | Frequency (%) | Field | Total | Frequency (%) | Field | Total | Frequency (%) |
| Benton | 10 | 21 | 48 | 3 | 21 | 14 | 8 | 21 | 38 |
| Clackamas | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Linn | 2 | 8 | 25 | 3 | 8 | 38 | 3 | 8 | 38 |
| Marion | 8 | 18 | 44 | 3 | 18 | 17 | 7 | 18 | 39 |
| Polk | 4 | 8 | 50 | 1 | 8 | 13 | 3 | 8 | 38 |
| Washington | 4 | 9 | 44 | 3 | 9 | 33 | 2 | 9 | 22 |
| Yamhill | 3 | 10 | 30 | 3 | 10 | 30 | 4 | 10 | 40 |

†: high (20≥ plants/m$^2$), medium (10 to 19 plants/m$^2$) and low (1 to 9 plants/m$^2$).

Table A-7 Frequency of resistance to all tested herbicide and number of fields surveyed for tree crop.

| Crop: | Tree crops | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **High presence†** | | | **Low presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 5 | 5 | 100 | 0 | 5 | 0 | 0 | 5 | 0 |
| Pinoxaden | 4 | 5 | 80 | 1 | 5 | 20 | 0 | 5 | 0 |
| Quizalofop-P-ethyl | 4 | 5 | 80 | 0 | 5 | 0 | 1 | 5 | 20 |
| Glyphosate | 2 | 5 | 40 | 3 | 5 | 60 | 0 | 5 | 0 |
| Glufosinate | 4 | 5 | 80 | 0 | 5 | 0 | 1 | 5 | 20 |
| Paraquat | 3 | 5 | 60 | 2 | 5 | 40 | 0 | 5 | 0 |
| Mesosulfuron | 1 | 5 | 20 | 3 | 5 | 60 | 1 | 5 | 20 |
| Pyroxsulam | 3 | 5 | 60 | 1 | 5 | 20 | 1 | 5 | 20 |
| Flufenacet + Metribuzin | 4 | 5 | 80 | 1 | 5 | 20 | 0 | 5 | 0 |
| Pronamide | 5 | 5 | 100 | 0 | 5 | 0 | 0 | 5 | 0 |
| Pyroxasulfone | 5 | 5 | 100 | 0 | 5 | 0 | 0 | 5 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-8. Frequency of resistance to all tested herbicide and number of fields surveyed for clover crop.

| Crop: | Clover | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **High presence†** | | | **Low presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 0 | 1 | 0 | 1 | 1 | 100 | 0 | 1 | 0 |
| Pinoxaden | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 100 |
| Quizalofop-P-ethyl | 0 | 1 | 0 | 1 | 1 | 100 | 0 | 1 | 0 |
| Glyphosate | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Glufosinate | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Paraquat | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Mesosulfuron | 0 | 1 | 0 | 1 | 1 | 100 | 0 | 1 | 0 |
| Pyroxsulam | 0 | 1 | 0 | 1 | 1 | 100 | 0 | 1 | 0 |
| Flufenacet + Metribuzin | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Pronamide | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 100 |
| Pyroxasulfone | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-9 Frequency of resistance to all tested herbicide and number of fields surveyed for oat crop.

| Crop: | Oat | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **High presence†** | | | **Low presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Pinoxaden | 0 | 1 | 0 | 1 | 1 | 100 | 0 | 1 | 0 |
| Quizalofop-P-ethyl | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 100 |
| Glyphosate | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 100 |
| Glufosinate | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Paraquat | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Mesosulfuron | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 100 |
| Pyroxsulam | 0 | 1 | 0 | 1 | 1 | 100 | 0 | 1 | 0 |
| Flufenacet + Metribuzin | 0 | 1 | 0 | 1 | 1 | 100 | 0 | 1 | 0 |
| Pronamide | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Pyroxasulfone | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-10 Frequency of resistance to all tested herbicide and number of fields surveyed for orchardgrass crop.

| Crop: | Orchardgrass | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **High presence†** | | | **Low presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 6 | 10 | 60 | 2 | 10 | 20 | 2 | 10 | 20 |
| Pinoxaden | 5 | 10 | 50 | 2 | 10 | 20 | 3 | 10 | 30 |
| Quizalofop-P-ethyl | 4 | 10 | 40 | 2 | 10 | 20 | 4 | 10 | 40 |
| Glyphosate | 5 | 10 | 50 | 4 | 10 | 40 | 1 | 10 | 10 |
| Glufosinate | 9 | 10 | 90 | 0 | 10 | 0 | 1 | 10 | 10 |
| Paraquat | 9 | 10 | 90 | 0 | 10 | 0 | 1 | 10 | 10 |
| Mesosulfuron | 2 | 10 | 20 | 2 | 10 | 20 | 6 | 10 | 60 |
| Pyroxsulam | 4 | 10 | 40 | 2 | 10 | 20 | 4 | 10 | 40 |
| Flufenacet + Metribuzin | 8 | 10 | 80 | 0 | 10 | 0 | 2 | 10 | 20 |
| Pronamide | 8 | 10 | 80 | 0 | 10 | 0 | 2 | 10 | 20 |
| Pyroxasulfone | 10 | 10 | 100 | 0 | 10 | 0 | 0 | 10 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-11 Frequency according to level of resistance for all tested herbicides at fields located in Benton County.

| County: | Benton | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **Low presence†** | | | **High presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 16 | 21 | 76 | 4 | 21 | 19 | 1 | 21 | 5 |
| Pinoxaden | 12 | 21 | 57 | 4 | 21 | 19 | 5 | 21 | 24 |
| Quizalofop-P-ethyl | 11 | 21 | 52 | 2 | 21 | 10 | 8 | 21 | 38 |
| Glyphosate | 14 | 21 | 67 | 2 | 21 | 10 | 5 | 21 | 24 |
| Glufosinate | 21 | 21 | 100 | 0 | 21 | 0 | 0 | 21 | 0 |
| Paraquat | 18 | 21 | 86 | 3 | 21 | 14 | 0 | 21 | 0 |
| Mesosulfuron | 5 | 21 | 24 | 11 | 21 | 52 | 5 | 21 | 24 |
| Pyroxsulam | 12 | 21 | 57 | 5 | 21 | 24 | 4 | 21 | 19 |
| Flufenacet + Metribuzin | 12 | 21 | 57 | 5 | 21 | 24 | 4 | 21 | 19 |
| Pronamide | 17 | 21 | 81 | 4 | 21 | 19 | 0 | 21 | 0 |
| Pyroxasulfone | 21 | 21 | 100 | 0 | 21 | 0 | 0 | 21 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-12 Frequency according to level of resistance for all tested herbicides at fields located in Clackamas County.

| County: | Clackamas | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **Low presence†** | | | **High presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Pinoxaden | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Quizalofop-P-ethyl | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Glyphosate | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Glufosinate | 0 | 1 | 0 | 1 | 1 | 100 | 0 | 1 | 0 |
| Paraquat | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 100 |
| Mesosulfuron | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 100 |
| Pyroxsulam | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Flufenacet + Metribuzin | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Pronamide | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Pyroxasulfone | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-13. Frequency according to level of resistance for all tested herbicides at fields located in Linn County.

| County: | Linn | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **Low presence†** | | | **High presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 5 | 8 | 63 | 2 | 8 | 25 | 1 | 8 | 13 |
| Pinoxaden | 5 | 8 | 63 | 1 | 8 | 13 | 2 | 8 | 25 |
| Quizalofop-P-ethyl | 5 | 8 | 63 | 2 | 8 | 25 | 1 | 8 | 13 |
| Glyphosate | 3 | 8 | 38 | 1 | 8 | 13 | 4 | 8 | 50 |
| Glufosinate | 8 | 8 | 100 | 0 | 8 | 0 | 0 | 8 | 0 |
| Paraquat | 8 | 8 | 100 | 0 | 8 | 0 | 0 | 8 | 0 |
| Mesosulfuron | 1 | 8 | 13 | 1 | 8 | 13 | 6 | 8 | 75 |
| Pyroxsulam | 2 | 8 | 25 | 1 | 8 | 13 | 5 | 8 | 63 |
| Flufenacet + Metribuzin | 6 | 8 | 75 | 0 | 8 | 0 | 2 | 8 | 25 |
| Pronamide | 8 | 8 | 100 | 0 | 8 | 0 | 0 | 8 | 0 |
| Pyroxasulfone | 8 | 8 | 100 | 0 | 8 | 0 | 0 | 8 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-14 Frequency according to level of resistance for all tested herbicides at fields located in Marion County.

| County: | Marion | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **Low presence†** | | | **High presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 12 | 18 | 67 | 2 | 18 | 11 | 4 | 18 | 22 |
| Pinoxaden | 7 | 18 | 39 | 3 | 18 | 17 | 8 | 18 | 44 |
| Quizalofop-ethyl | 6 | 18 | 33 | 6 | 18 | 33 | 6 | 18 | 33 |
| Glyphosate | 10 | 18 | 56 | 3 | 18 | 17 | 5 | 18 | 28 |
| Glufosinate | 17 | 18 | 94 | 1 | 18 | 6 | 0 | 18 | 0 |
| Paraquat | 18 | 18 | 100 | 0 | 18 | 0 | 0 | 18 | 0 |
| Mesosulfuron | 3 | 18 | 17 | 8 | 18 | 44 | 7 | 18 | 39 |
| Pyroxsulam | 7 | 18 | 39 | 3 | 18 | 17 | 8 | 18 | 44 |
| Flufenacet + Metribuzin | 14 | 18 | 78 | 3 | 18 | 17 | 1 | 18 | 6 |
| Pronamide | 16 | 18 | 89 | 2 | 18 | 11 | 0 | 18 | 0 |
| Pyroxasulfone | 18 | 18 | 100 | 0 | 18 | 0 | 0 | 18 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-15 Frequency according to level of resistance for all tested herbicides at fields located in Polk County.

| County: | Polk | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **Low presence†** | | | **High presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 7 | 8 | 88 | 1 | 8 | 13 | 0 | 8 | 0 |
| Pinoxaden | 3 | 8 | 38 | 3 | 8 | 38 | 2 | 8 | 25 |
| Quizalofop-ethyl | 3 | 8 | 38 | 2 | 8 | 25 | 3 | 8 | 38 |
| Glyphosate | 5 | 8 | 63 | 2 | 8 | 25 | 1 | 8 | 13 |
| Glufosinate | 8 | 8 | 100 | 0 | 8 | 0 | 0 | 8 | 0 |
| Paraquat | 6 | 8 | 75 | 1 | 8 | 13 | 1 | 8 | 13 |
| Mesosulfuron | 2 | 8 | 25 | 4 | 8 | 50 | 2 | 8 | 25 |
| Pyroxsulam | 5 | 8 | 63 | 1 | 8 | 13 | 2 | 8 | 25 |
| Flufenacet + Metribuzin | 5 | 8 | 63 | 2 | 8 | 25 | 1 | 8 | 13 |
| Pronamide | 7 | 8 | 88 | 1 | 8 | 13 | 0 | 8 | 0 |
| Pyroxasulfone | 8 | 8 | 100 | 0 | 8 | 0 | 0 | 8 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-16 Frequency according to level of resistance for all tested herbicides at fields located in Washington County.

| County: | Washington | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **Low presence†** | | | **High presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 4 | 9 | 44 | 4 | 9 | 44 | 1 | 9 | 11 |
| Pinoxaden | 0 | 9 | 0 | 3 | 9 | 33 | 6 | 9 | 67 |
| Quizalofop-ethyl | 0 | 9 | 0 | 1 | 9 | 11 | 8 | 9 | 89 |
| Glyphosate | 5 | 9 | 56 | 3 | 9 | 33 | 1 | 9 | 11 |
| Glufosinate | 9 | 9 | 100 | 0 | 9 | 0 | 0 | 9 | 0 |
| Paraquat | 9 | 9 | 100 | 0 | 9 | 0 | 0 | 9 | 0 |
| Mesosulfuron | 0 | 9 | 0 | 1 | 9 | 11 | 8 | 9 | 89 |
| Pyroxsulam | 1 | 9 | 11 | 3 | 9 | 33 | 5 | 9 | 56 |
| Flufenacet + Metribuzin | 1 | 9 | 11 | 4 | 9 | 44 | 4 | 9 | 44 |
| Pronamide | 6 | 9 | 67 | 3 | 9 | 33 | 0 | 9 | 0 |
| Pyroxasulfone | 9 | 9 | 100 | 0 | 9 | 0 | 0 | 9 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-17 Frequency according to level of resistance for all tested herbicides at fields located in Yamhill County.

| **County:** | **Yamhill** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Resistance level:** | **Susceptible†** | | | **Low presence†** | | | **High presence†** | | |
| **Herbicide** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Clethodim | 6 | 10 | 60 | 3 | 10 | 30 | 1 | 10 | 10 |
| Pinoxaden | 4 | 10 | 40 | 1 | 10 | 10 | 5 | 10 | 50 |
| Quizalofop-ethyl | 3 | 10 | 30 | 3 | 10 | 30 | 4 | 10 | 40 |
| Glyphosate | 5 | 10 | 50 | 0 | 10 | 0 | 5 | 10 | 50 |
| Glufosinate | 10 | 10 | 100 | 0 | 10 | 0 | 0 | 10 | 0 |
| Paraquat | 9 | 10 | 90 | 1 | 10 | 10 | 0 | 10 | 0 |
| Mesosulfuron | 1 | 10 | 10 | 4 | 10 | 40 | 5 | 10 | 50 |
| Pyroxsulam | 1 | 10 | 10 | 6 | 10 | 60 | 3 | 10 | 30 |
| Flufenacet + Metribuzin | 8 | 10 | 80 | 1 | 10 | 10 | 1 | 10 | 10 |
| Pronamide | 10 | 10 | 100 | 0 | 10 | 0 | 0 | 10 | 0 |
| Pyroxasulfone | 9 | 10 | 90 | 1 | 10 | 10 | 0 | 10 | 0 |

†: High presence (20% or more of survival and less than 70% of green area reduction), low presence (2-19% of survival and green area reduction of between 70 to 90%) and susceptible (less than 2% of survival and green area reduction of at least 90%).

Table A-18 Frequency of single resistance and susceptible cases on each county in the surveyed area.

| | Susceptible | | | Single | | |
|---|---|---|---|---|---|---|
| **County** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Benton | 4 | 21 | 19 | 0 | 21 | 0 |
| Clackamas | 0 | 1 | 0 | 0 | 1 | 0 |
| Linn | 1 | 8 | 13 | 1 | 8 | 13 |
| Marion | 3 | 18 | 17 | 0 | 18 | 0 |
| Polk | 1 | 8 | 13 | 1 | 8 | 13 |
| Washington | 0 | 9 | 0 | 0 | 9 | 0 |
| Yamhill | 1 | 10 | 10 | 0 | 10 | 0 |

Table A-19 Frequency multiple, cross resistance and the combination cases on each county in the surveyed area.

| | Multiple | | | Cross | | | Cross & Multiple | | |
|---|---|---|---|---|---|---|---|---|---|
| **County** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** | **Fields** | **Total** | **Frequency (%)** |
| Benton | 9 | 21 | 43 | 0 | 21 | 0 | 8 | 21 | 38 |
| Clackamas | 1 | 1 | 100 | 0 | 1 | 0 | 0 | 1 | 0 |
| Linn | 0 | 8 | 0 | 1 | 8 | 13 | 5 | 8 | 62 |
| Marion | 1 | 18 | 6 | 1 | 18 | 6 | 13 | 18 | 78 |
| Polk | 3 | 8 | 38 | 0 | 8 | 0 | 4 | 8 | 50 |
| Washington | 0 | 9 | 0 | 0 | 9 | 0 | 9 | 9 | 100 |
| Yamhill | 0 | 10 | 30 | 2 | 10 | 20 | 7 | 10 | 70 |

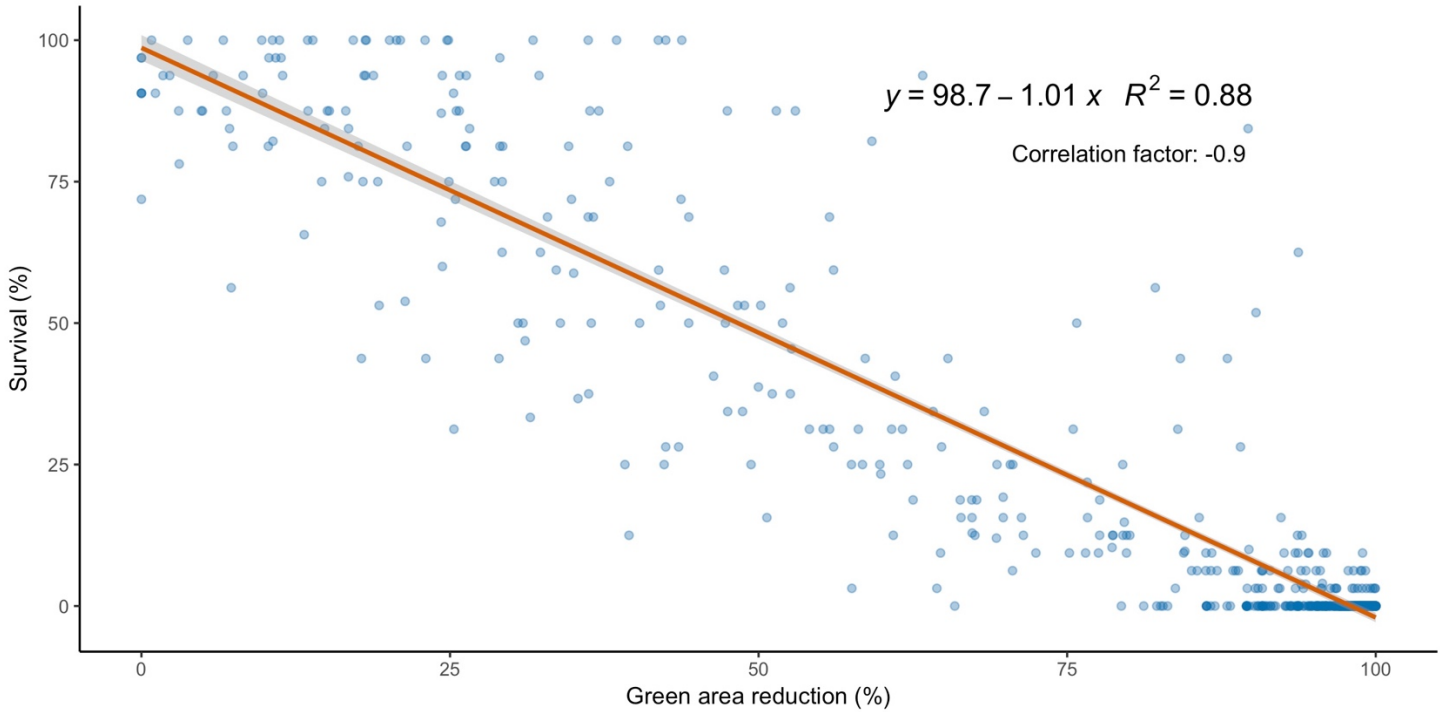



Figure A-2. Correlation plot between Survival rate and green area reduction percentage measurements

```
//HERBICIDE INJURY GREEN AREA ANALYSIS .imj file - IMAGEJ - JUNE 2019

                // LUCAS KOPECKY BOBADILLA - OREGON STATE UNIVERSITY


                //BEFORE RUNING THIS MACRO:
                //1 - Add a universal scale based in your scale photo and make it
                universal
                //2 - Run the color threshold method manually using  IsoData as
                method, and Lab color space
                //3 - set the numbers of the colour space (make sure to select a
                measure that captures only the green area)
                //4 - Then run a measure of the selected area
                //5 - change in the indited location in the code with the numbers used
                in the colour space
                //6 - change the file name to be generated and the input and output
                locations


                function seedlings(input, output, filename){
                open(input + "/" + filename);
                // Colour Thresholding-------------
                run("Color Threshold...");
                min=newArray(3);
                max=newArray(3);
                filter=newArray(3);
                a=getTitle();
                run("HSB Stack");
                run("Convert Stack to Images");
                selectWindow("Hue");
                rename("0");
                selectWindow("Saturation");
                rename("1");
                selectWindow("Brightness");
                rename("2");
                \\CHANGE L VALUES:
                min[0]=47;
                max[0]=117;
                filter[0]="pass";
                \\CHANGE a VALUES:
                min[1]=33;
                max[1]=255;
```

```
filter[1]="pass";
\\CHANGE b VALUES:
min[2]=12;
max[2]=255;
filter[2]="pass";
for (i=0;i<3;i++){
  selectWindow(""+i);
  setThreshold(min[i], max[i]);
  run("Convert to Mask");
  if (filter[i]=="stop")  run("Invert");
}
imageCalculator("AND create", "0","1");
imageCalculator("AND create", "Result of 0","2");
for (i=0;i<3;i++){
  selectWindow(""+i);
  close();
}
selectWindow("Result of 0");
close();
selectWindow("Result of Result of 0");
rename(a);
// Colour Thresholding-------------
run("Measure");
close();
}
//ADD YOUR FILE NAME, INPUT AND OUTPUT
filename = "glyphosate_2018";
input = "/Volumes/files_500gb/2 -
GRAD_SCHOOL/SURVEY/2018/GREENHOUSE/Glyphosate/analysis"; \\FOLDER
LOCATION WITH THE IMAGES
output = "/Volumes/files_500gb/2 -
GRAD_SCHOOL/SURVEY/2018/GREENHOUSE/Glyphosate/analysis"; \\PLACE TO
SAVE THE CSV FILE


\\ loop of the above function:
list = getFileList(input);
for (i = 0; i < list.length; i++){
        seedlings(input, output, list[i]);
}       //get list of all images in the folder
saveAs("Results", filename + ".csv");
```

# APPENDIX B

## Characterization of populations for herbicide resistance

Italian ryegrass is an obligate outcrossing species; therefore, in order to greater homozygous level of resistance, 100 seeds collected from the fields of each population w grown in the greenhouse in 267 ml plastic pots containing commercial potting mix (Sunshine mix 1 Potting mix, Sun Gro Horticulture, Inc., 110$^{th}$ Ave. NE, Suite 490, Bellevue, WA 98004) to produce seeds. Once the plants started to produce tillers, those was separated in different plastic pots to produce clones. After five days of establishment and acclimation of the tiller-clones, one of the tillers of each plant were sprayed at recommended field rate. To selected plants with the resistant trait, plants of the PR, RD and TF population were respectively treated with glyphosate (Makaze, 0.840 kg ae ha$^{-1}$; Loveland Products, Inc., 3005 Rocky Mountain Avenue Loveland, CO 80538 United States ), pinoxaden (Axial XL 0.603 kg ae ha$^{-1}$; Syngenta Crop Protection AG, Basel, Switzerland.) and pyroxsulam (Powerflex 0.600 kg ae ha$^{-1}$ ; Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268); FG population was treated with all three herbicides. Twenty-one days after application on the tillers, the not sprayed clones from the plants that survived were separated from the ones that did not survive to avoid cross-pollination and produce seeds. Seeds were harvested at maturity five weeks after flowering. These second generations of seeds were used in the dose-response experiment. Seeds were kept for three months in dark and cold environment and germinated on four germination boxes with size of 11x11x2.8 cm (156C container, Hoffman Manufacturing Inc; Corvallis, OR 97330 USA) with a square blue paper blotters in a growth chamber set with a photoperiod of 16h and a temperature regime of 21/10 °C for 10 days until

seedling formation.

The dose-response experiment was conducted using a randomized complete blocks design with seven herbicide treatments plus a control. Four square plastic trays of 24 by 24 cm with commercial potting mix (Sunshine mix 1 Potting mix, Sun Gro Horticulture, Inc., 110$^{th}$ Ave. NE, Suite 490, Bellevue, WA 98004) were used as blocks for each treatment where nine seedlings were placed on each tray. Plants were treated when they reach the two-leaf stage and have 10 to 16 cm of height. The equipment used was an air cabinet sprayer (Generation III Spray Chamber, De Vries Manufacturing, 86956 State HWY 251, Hollandale, MN 56054) with a flat-fan spray *Teejet nozzle 8004* set to deliver 187 L ha$^{-1}$. Survival percentage and biomass were collected 21 days after treatment. PR, TF, and RD populations were treated respectively with glyphosate (Makaze®; Loveland Products, Inc., 3005 Rocky Mountain Avenue Loveland, CO 80538 United States) at the rates 0, 0.105, 0.210, 0.420, 0.840, 1.680, 3.360, 6.700 and 13.400 kg ae ha$^{-1}$; pyroxsulam (Powerflex®; Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, IN 46268) at the rates 0, 0.075, 0.15, 0.3, 0.6, 1.2, 2.4, 4.8 and 9.6 kg ae ha$^{-1}$ ; and Select Max (Select Max® Herbicide with Inside Technology is a registered trademark of Valent U.S.A. LLC and "with Inside Technology" is a trademark of Valent U.S.A. LLC) at the rates 0.000, 0.170, 0.340, 0.680, 1.360, 2.720, 5.440, 10.880 and 21.760 kg ai/ha. The FG population was used as a susceptible check. A nonlinear log-logistic model [1] was used to characterize the response and both $ED_{50}$ (dose necessary to reduce 50 % of biomass and kill 50% of the plants tested), were computed (Table B.1 to B.3).

$$y = c + \frac{d - c}{1 + \left(\frac{x}{ED_{50}}\right)^{b}} \qquad [1]$$

Where *y* is the response, *d* refers to the upper limit and *c* to the lower limit of the sigmoid curve, $ED_{50}$ denotes the dose, x refers to the necessary rate to reduce 50% of biomass or kill 50% of the plants between *d* and *c,* and *b* refers to the relative slope of the curve around $ED_{50}$ and $LD_{50}$. The experiment was repeated to confirm results. Glyphosate (Figure B.1, B.2) and clethodim (Figure B.3, B.4) dose responses were analyzed using a four parameter log-logistic while pyroxsulam dose response was analyzed using a three parameter log-logistic. Data were transformed using a Box-Cox transformation when needed to meet homogeneity and normality assumptions.

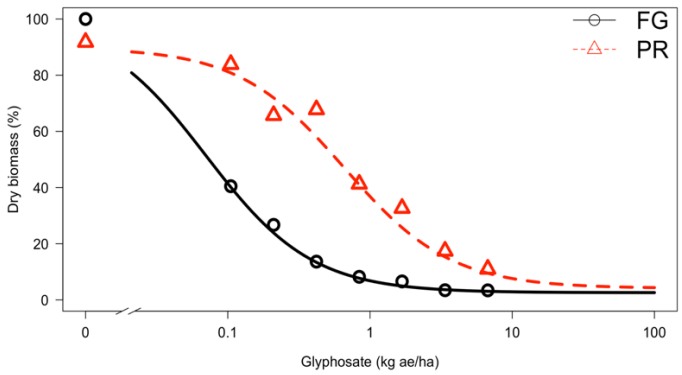



Figure B-1. Dose response curve for dry biomass reduction in response to glyphosate testing the biotypes FG (black line and circles) and PR (red line and triangles).

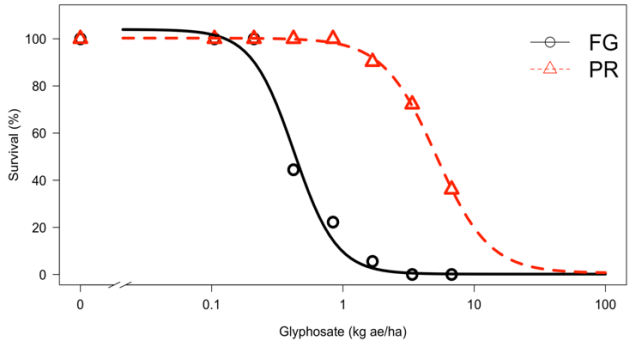


Figure B-2. Dose response curve for survival percentage in response to glyphosate testing the biotypes FG (black line and circles) and PR (red line and triangles).

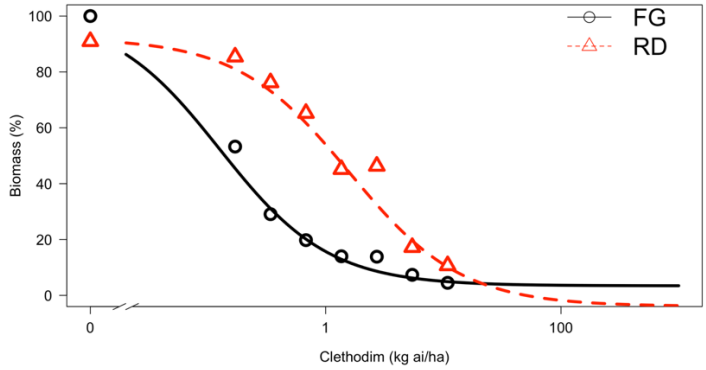



Figure B-3.Dose response curve for dry biomass reduction in response to clethodim testing the biotypes FG (black line and circles) and RD (red line and triangles).

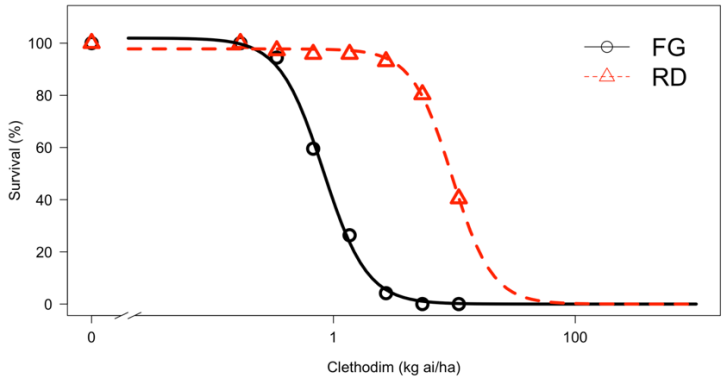



Figure B-4. Dose response curve for survival percentage in response to clethodim testing the biotypes FG (black line and circles) and RD (red line and triangles).

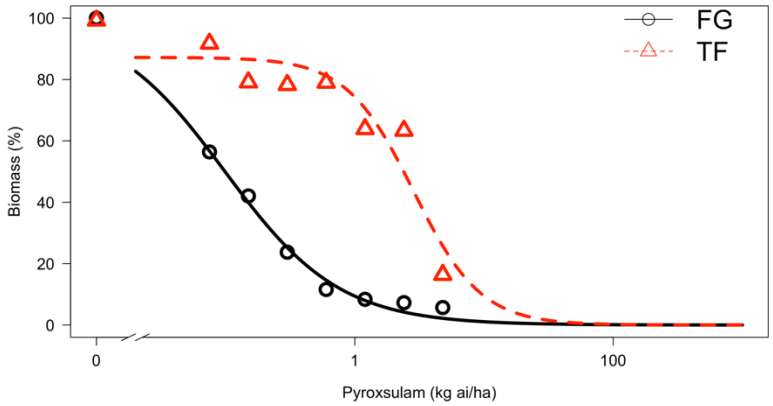


Figure B-5. Dose response curve for dry biomass reduction in response to pyroxsulam testing the biotypes FG (black line and circles) and TF (red line and triangles).

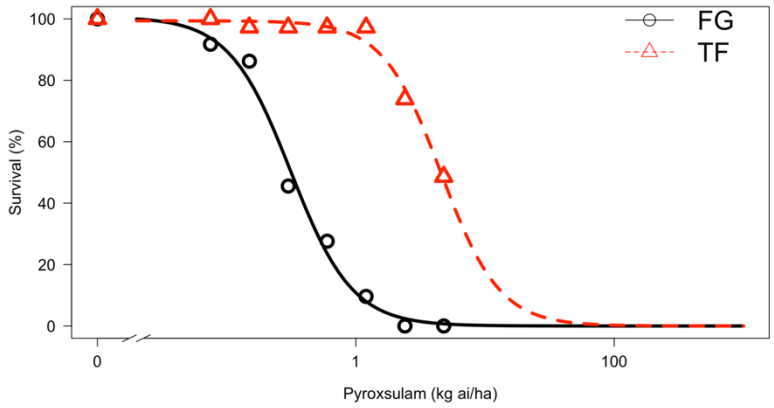



Figure B-6. Dose response curve for survival percentage in response to pyroxsulam testing the biotypes FG (black line and circles) and TF (red line and triangles).

Table B-1. Summary table of results of a log-logistics parameter fitted for glyphosate dose response

| Survival (%) | | | | | | |
|---|---|---|---|---|---|---|
| **Biotype** | **Herbicide** | **Slope** | **Lower limit** | **Upper Limit** | **ED-50** | **ED-50 ratio** |
| FG | Glyphosate | 2.68 (±0.32) | 0.17 (±2.04) | 100.00 (±2.01) | 0.42 (±0.02) | - |
| PR | | 2.15 (±0.60) | 0.61 (±4.00) | 100.00 (±1.40) | 5.13 (±1.83) | 12.21 |
| **Biomass Reduction (%)** | | | | | | |
| **Biotype** | **Herbicide** | **Slope** | **Lower limit** | **Upper Limit** | **ED-50** | **ED-50 ratio** |
| FG | Glyphosate | 1.17 (±0.23) | 2.58 (±1.09) | 100.08 (±9.94) | 0.07 (±0.02) | - |
| PR | | 1.16 (±0.34) | 4.14 (±5.66) | 89.84 (±8.66) | 0.66 (±0.17) | 9.43 |

Table B-2. Summary table of results of a log-logistics parameter fitted for clethodim dose response

| Survival (%) | | | | | | |
|---|---|---|---|---|---|---|
| **Biotype** | **Herbicide** | **Slope** | **Lower limit** | **Upper Limit** | **ED-50** | **ED-50 ratio** |
| FG | Clethodim | 2.68 (±0.32) | 0.15 (±2.04) | 85.70 (±7.36) | 1.13 (±0.07) | - |
| RD | | 2.15 (±0.60) | 0.63 (±3.00) | 97.68 (±7.20) | 9.30 (±1.38) | 8.23 |
| **Biomass Reduction (%)** | | | | | | |
| **Biotype** | **Herbicide** | **Slope** | **Lower limit** | **Upper Limit** | **ED-50** | **ED-50 ratio** |
| FG | Clethodim | 0.94 (±0.26) | 3.45 (±2.55) | 91.05 (±9.42) | 0.13 (±0.04) | - |
| RD | | 0.91 (±0.29) | 3.92 (±2.57) | 92.13 (±8.98) | 1.58 (±0.62) | 12.15 |

Table B-3. Summary table of results of a log-logistics parameter fitted for pyroxsulam dose response

| Survival (%) | | | | | |
|---|---|---|---|---|---|
| **Biotype** | **Herbicide** | **Slope** | **Upper Limit** | **ED-50** | **ED-50 ratio** |
| FG | Pyroxsulam | 1.83 (±0.18) | 100.63 (±3.04) | 0.31 (±0.02) | - |
| TF | | 1.93 (±0.30) | 99.37 (±1.71) | 4.58 (±0.33) | 14.77 |
| **Biomass Reduction (%)** | | | | | |
| **Biotype** | **Herbicide** | **Slope** | **Upper Limit** | **ED-50** | **ED-50 ratio** |
| FG | Pyroxsulam | 0.98 (±0.21) | 100.09 (±6.50) | 0.10 (±0.02) | - |
| TF | | 1.68 (±0.78) | 87.21 (±5.07) | 2.85 (±0.47) | 28.50 |

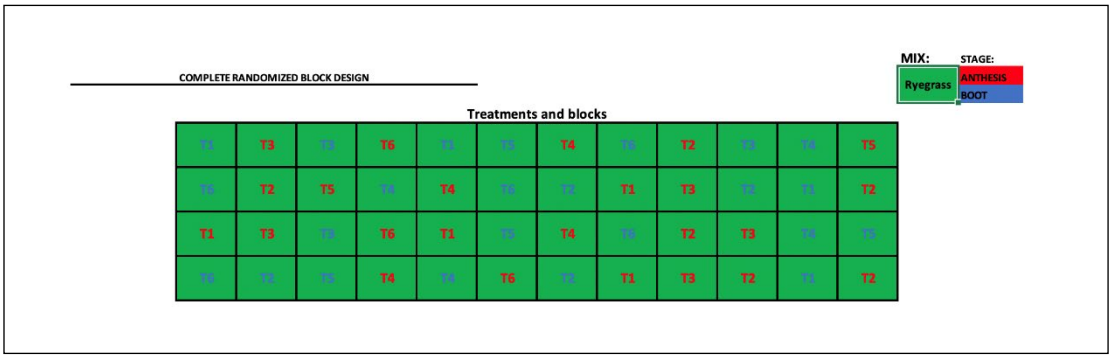



Figure B-7. Trial design at greenhouse 2017/2018 (greenhouse 2018 had three extra treatments but design was the same). Each square represents a plot with colour green representing the species and the letters represent the treatment colored according to growth stage

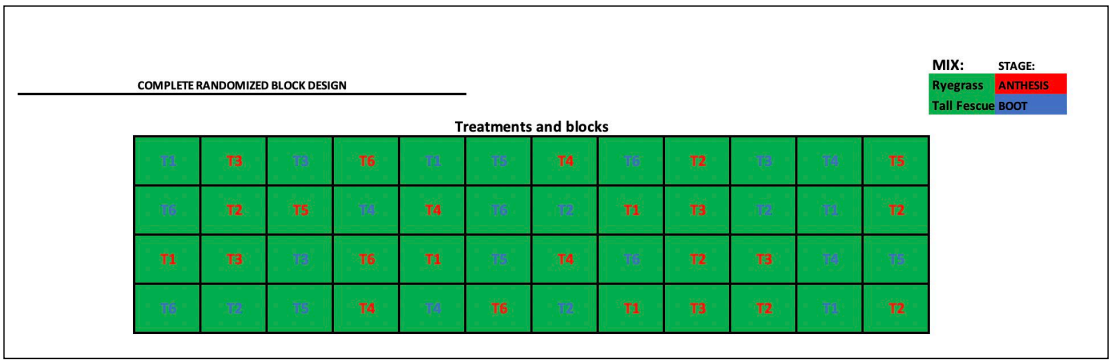



Figure B-8. Trial design at Hyslop and Schmidt farm trials (Schmith trial had three extra treatments but design was the same). Each square represents a plot with colour green representing the species and the letters represent the treatment colored according to growth stage

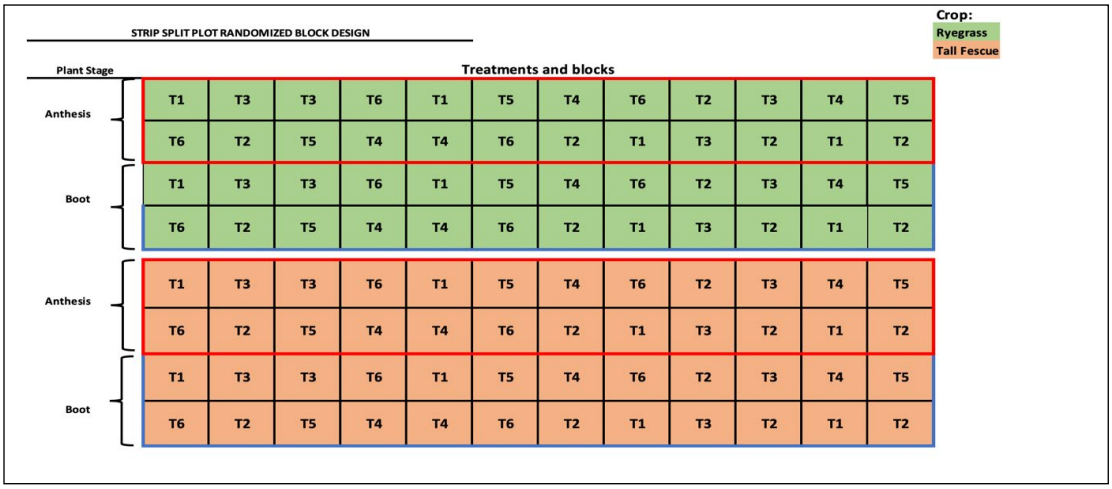



Figure B-9. Trial design at the Gaston and Dallas trial, OR (Gaston trial had three extra treatments, but design was the same). Each square represents a plot with colour green representing the species and the letters represent the treatment colored according to growth stage.

Table B-4. Analysis of deviance results for generalized linear mixed model on seed viability response for 2017 greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 652.059 | 5 | < 0.001 |
| Growth Stage | 6.459 | 1 | 0.011 |
| Biotype | 63.006 | 3 | < 0.001 |
| Treatment vs Growth Stage | 5.157 | 5 | 0.397 |
| Treatment vs Biotype | 63.483 | 15 | < 0.001 |
| Growth Stage vs Biotype | 2.747 | 3 | 0.432 |
| Treatment vs Growth Stage vs Biotype | 21.900 | 15 | 0.110 |

Table B-5. Analysis of deviance results for generalized linear mixed model on seed viability response for 2018 greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 2335.243 | 8 | < 0.001 |
| Growth Stage | 11.288 | 1 | < 0.001 |
| Biotype | 36.360 | 3 | < 0.001 |
| Treatment vs Growth Stage | 356.069 | 8 | < 0.001 |
| Treatment vs Biotype | 344.087 | 24 | < 0.001 |
| Growth Stage vs Biotype | 4.444 | 3 | 0.217 |
| Treatment vs Growth Stage vs Biotype | 76.305 | 24 | < 0.001 |

Table B-6. Analysis of deviance results for generalized linear mixed model on seed weight response for 2017 greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 516.208 | 5 | < 0.001 |
| Growth Stage | 0.788 | 1 | 0.375 |
| Biotype | 27.175 | 3 | < 0.001 |
| Treatment vs Growth Stage | 9.593 | 5 | 0.088 |
| Treatment vs Biotype | 27.454 | 15 | 0.025 |
| Growth Stage vs Biotype | 10.644 | 3 | 0.014 |
| Treatment vs Growth Stage vs Biotype | 26.346 | 15 | 0.035 |

Table B-7. Analysis of deviance results for generalized linear mixed model on seed weight response for 2018 greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 414.887 | 8 | < 0.001 |
| Growth Stage | 0.007 | 1 | 0.933 |
| Biotype | 6.157 | 3 | 0.104 |
| Treatment vs Growth Stage | 7.130 | 8 | 0.523 |
| Treatment vs Biotype | 30.066 | 24 | 0.183 |
| Growth Stage vs Biotype | 3.469 | 3 | 0.325 |
| Treatment vs Growth Stage vs Biotype | 19.653 | 24 | 0.716 |

Table B-8. Analysis of deviance results for generalized linear mixed model on seed viability reduction after stress for 2017 greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 59.705 | 8 | < 0.001 |
| Growth Stage | 20.707 | 4 | < 0.001 |
| Biotype | 216.105 | 5 | < 0.001 |
| Treatment vs Growth Stage | 27.895 | 7 | < 0.001 |
| Treatment vs Biotype | 71.872 | 17 | < 0.001 |
| Growth Stage vs Biotype | 27.740 | 5 | < 0.001 |
| Treatment vs Growth Stage vs Biotype | 37.663 | 15 | 0.001 |

Table B-9. Analysis of deviance results for generalized linear mixed model on seed viability reduction after stress for 2018 greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 39.805 | 9 | < 0.001 |
| Growth Stage | 1.537 | 1 | 0.215 |
| Biotype | 440.499 | 4 | < 0.001 |
| Treatment vs Growth Stage | 48.017 | 8 | < 0.001 |
| Treatment vs Biotype | 107.808 | 24 | < 0.001 |
| Growth Stage vs Biotype | 3.216 | 3 | 0.360 |
| Treatment vs Growth Stage vs Biotype | 40.140 | 24 | 0.021 |

Table B-10. Analysis of deviance results for generalized linear mixed model on pollen viability reduction after stress for 2017 greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 3.250 | 5 | 0.662 |
| Growth Stage | 0.786 | 1 | 0.375 |
| Biotype | 11.013 | 3 | 0.012 |
| Treatment vs Growth Stage | 8.248 | 5 | 0.143 |
| Treatment vs Biotype | 37.285 | 15 | 0.211 |
| Growth Stage vs Biotype | 3.343 | 3 | 0.342 |
| Treatment vs Growth Stage vs Biotype | 17.17 | 15 | 0.309 |

Table B-11. Analysis of deviance results for generalized linear mixed model on pollen viability reduction after stress for 2018 greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 12.399 | 8 | 0.134 |
| Growth Stage | 0.233 | 1 | 0.629 |
| Biotype | 1.266 | 3 | 0.737 |
| Treatment vs Growth Stage | 8.728 | 8 | 0.366 |
| Treatment vs Biotype | 23.056 | 24 | 0.516 |
| Growth Stage vs Biotype | 2.744 | 3 | 0.433 |
| Treatment vs Growth Stage vs Biotype | 43.326 | 24 | 0.239 |

Table B-12. Analysis of deviance results for generalized linear mixed model on seed viability response for pulled data from greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 1312.762 | 5 | < 0.001 |
| Growth Stage | 11.926 | 1 | < 0.001 |
| Biotype | 36.648 | 3 | < 0.001 |
| Treatment vs Growth Stage | 81.563 | 5 | < 0.001 |
| Treatment vs Biotype | 93.392 | 15 | < 0.001 |
| Growth Stage vs Biotype | 1.905 | 3 | 0.592 |
| Treatment vs Growth Stage vs Biotype | 19.541 | 15 | 0.190 |

Table B-13. Analysis of deviance results for generalized linear mixed model on seed viability reduction after stress for pulled data of greenhouse trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 11.654 | 5 | 0.050 |
| Growth Stage | 0.377 | 1 | 0.539 |
| Biotype | 315.542 | 3 | < 0.001 |
| Treatment vs Growth Stage | 3.312 | 5 | 0.652 |
| Treatment vs Biotype | 2.392 | 15 | 0.566 |
| Growth Stage vs Biotype | 0.461 | 3 | 0.927 |
| Treatment vs Growth Stage vs Biotype | 19.136 | 15 | 0.208 |

Table B-14. Analysis of deviance results for generalized linear mixed model on seed viability response for Dallas, OR trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 1.968 | 4 | 0.742 |
| Growth Stage | 0.110 | 1 | 0.740 |
| Species | 641.503 | 1 | < 0.001 |
| Treatment vs Growth Stage | 1.844 | 4 | 0.764 |
| Treatment vs Species | 9.027 | 4 | 0.060 |
| Growth Stage vs Species | 0.121 | 1 | 0.728 |
| Treatment vs Growth Stage vs Species | 2.158 | 4 | 0.707 |

Table B-15. Analysis of deviance results for generalized linear mixed model on seed viability response for Hyslop farm trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 13932.242 | 5 | < 0.001 |
| Growth Stage | 8.271 | 1 | 0.004 |
| Species | 29.623 | 1 | < 0.001 |
| Treatment vs Growth Stage | 248.689 | 5 | < 0.001 |
| Treatment vs Species | 1626.920 | 5 | < 0.001 |
| Growth Stage vs Species | 0.091 | 1 | 0.763 |
| Treatment vs Growth Stage vs Species | 16.812 | 5 | 0.005 |

Table B-16. Analysis of deviance results for generalized linear mixed model on seed viability response for Gaston, OR trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 7000.144 | 8 | < 0.001 |
| Growth Stage | 42.901 | 1 | < 0.001 |
| Species | 20.665 | 1 | < 0.001 |
| Treatment vs Growth Stage | 538.839 | 8 | < 0.001 |
| Treatment vs Species | 295.792 | 8 | < 0.001 |
| Growth Stage vs Species | 0.002 | 1 | 0.966 |
| Treatment vs Growth Stage vs Species | 180.433 | 8 | < 0.001 |

Table B-17. Analysis of deviance results for generalized linear mixed model on seed viability response for Schmidt farm trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 25573.690 | 8 | < 0.001 |
| Growth Stage | 15.395 | 1 | < 0.001 |
| Species | 397.579 | 1 | < 0.001 |
| Treatment vs Growth Stage | 493.394 | 8 | < 0.001 |
| Treatment vs Species | 772.501 | 8 | < 0.001 |
| Growth Stage vs Species | 23.808 | 1 | < 0.001 |
| Treatment vs Growth Stage vs Species | 649.881 | 8 | < 0.001 |

Table B-18. Analysis of deviance results for generalized linear mixed model on seed weight response for Dallas, OR trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 5.960 | 4 | 0.202 |
| Growth Stage | 0.000 | 1 | 0.987 |
| Species | 112.651 | 1 | < 0.001 |
| Treatment vs Growth Stage | 0.000 | 4 | 1.000 |
| Treatment vs Species | 6.082 | 4 | 0.193 |
| Growth Stage vs Species | 0.000 | 1 | 0.967 |
| Treatment vs Growth Stage vs Species | 0.000 | 4 | 1.000 |

Table B-19. Analysis of deviance results for generalized linear mixed model on seed weight response for Hyslop farm trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 765.128 | 5 | < 0.001 |
| Growth Stage | 0.528 | 1 | 0.468 |
| Species | 23.549 | 1 | < 0.001 |
| Treatment vs Growth Stage | 0.800 | 5 | 0.977 |
| Treatment vs Species | 133.011 | 5 | < 0.001 |
| Growth Stage vs Species | 0.103 | 1 | 0.749 |
| Treatment vs Growth Stage vs Species | 1.522 | 5 | 0.910 |

Table B-20. Analysis of deviance results for generalized linear mixed model on seed weight response for Gaston, OR trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 1015.164 | 8 | < 0.001 |
| Growth Stage | 0.198 | 1 | 0.656 |
| Species | 2.938 | 1 | 0.086 |
| Treatment vs Growth Stage | 6.819 | 8 | 0.556 |
| Treatment vs Species | 10.935 | 8 | 0.205 |
| Growth Stage vs Species | 4.015 | 1 | 0.045 |
| Treatment vs Growth Stage vs Species | 8.585 | 8 | 0.379 |

Table B-21. Analysis of deviance results for generalized linear mixed model on seed weight response for Schmidt farm trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 5522.045 | 8 | < 0.001 |
| Growth Stage | 2.139 | 1 | 0.144 |
| Species | 0.001 | 1 | 0.971 |
| Treatment vs Growth Stage | 6.410 | 8 | 0.601 |
| Treatment vs Species | 65.424 | 8 | < 0.001 |
| Growth Stage vs Species | 2.173 | 1 | 0.140 |
| Treatment vs Growth Stage vs Species | 16.448 | 8 | 0.036 |

Table B-22. Analysis of deviance results for generalized linear mixed model on seed viability reduction response after stress for Dallas, OR trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 7.649 | 6 | 0.265 |
| Growth Stage | 0.872 | 3 | 0.832 |
| Species | 8.859 | 1 | 0.003 |
| Treatment vs Growth Stage | 8.433 | 4 | 0.077 |
| Treatment vs Species | 8.143 | 4 | 0.086 |
| Growth Stage vs Species | 0.048 | 1 | 0.826 |
| Treatment vs Growth Stage vs Species | 1.764 | 4 | 0.779 |

Table B-23. Analysis of deviance results for generalized linear mixed model on seed viability reduction response after stress for Hyslop farm trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 54.767 | 11 | < 0.001 |
| Growth Stage | 7.832 | 7 | 0.348 |
| Species | 13.227 | 4 | 0.010 |
| Treatment vs Growth Stage | 4.348 | 7 | 0.739 |
| Treatment vs Species | 13.617 | 7 | 0.008 |
| Growth Stage vs Species | 3.936 | 3 | 0.268 |
| Treatment vs Growth Stage vs Species | 2.317 | 5 | 0.804 |

Table B-24. Analysis of deviance results for generalized linear mixed model on seed viability reduction response after stress for Gaston, OR trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 27.975 | 10 | 0.002 |
| Growth Stage | 15.562 | 2 | < 0.001 |
| Species | 20.256 | 2 | < 0.001 |
| Treatment vs Growth Stage | 25.687 | 8 | 0.001 |
| Treatment vs Species | 25.302 | 8 | 0.001 |
| Growth Stage vs Species | 1.063 | 1 | 0.303 |
| Treatment vs Growth Stage vs Species | 17.450 | 8 | 0.026 |

Table B-25. Analysis of deviance results for generalized linear mixed model on seed viability reduction response after stress for Schmidt farm trial

| Variation | Wald chi-square | DF | *p*-value |
|---|---|---|---|
| Treatment | 185.133 | 9 | < 0.001 |
| Growth Stage | 1.324 | 1 | 0.250 |
| Species | 28.547 | 2 | < 0.001 |
| Treatment vs Growth Stage | 46.168 | 8 | < 0.001 |
| Treatment vs Species | 91.567 | 8 | < 0.001 |
| Growth Stage vs Species | 2.467 | 1 | 0.116 |
| Treatment vs Growth Stage vs Species | 13.671 | 8 | 0.091 |

Table B-26. Viability of seeds of four different Italian ryegrass biotypes after synthetic auxin herbicide treatment applied at two growth stages in greenhouse 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatment | Biotype | Viability (SE) | |
| | | ---------- % ---------- | |
| **Control** | **FG** | 74.2 (±3.7) c | 73.3 (±3.7) cd |
| **2,4-D** | | 78.2 (±3.8) c | 74.0 (±3.7) cd |
| **Dicamba** | | 71.8 (±3.7) c | 73.7 (±3.7) cd |
| **Aminopyralid** | | 29.2 (±2.3) a | 32.8 (±2.5) a |
| **2,4-D + Clopyralid** | | 76.5 (±3.8) c | 73.7 (±3.7) cd |
| **Dicamba + 2,4-D** | | 66.5 (±3.5) c | 70.3 (±3.6) cd |
| **Control** | **PR** | 76.2 (±3.8) c | 84.3 (±4.0) d |
| **2,4-D** | | 74.7 (±3.7) c | 81.5 (±3.9) d |
| **Dicamba** | | 74.7 (±3.7) c | 71.7 (±3.7) cd |
| **Aminopyralid** | | 46.8 (±3.0) b | 44.3 (±2.9) ab |
| **2,4-D + Clopyralid** | | 69.8 (±3.6) c | 75.8 (±3.8) cd |
| **Dicamba + 2,4-D** | | 75.8 (±3.8) c | 83.0 (±3.9) d |
| **Control** | **RD** | 84.7 (±4.0) c | 78.3 (±3.8) d |
| **2,4-D** | | 77.2 (±3.8) c | 76.2 (±3.8) d |
| **Dicamba** | | 86.8 (±4.0) c | 78.8 (±3.8) d |
| **Aminopyralid** | | 47.7 (±3.0) b | 57.3 (±3.3) bc |
| **2,4-D + Clopyralid** | | 75.5 (±3.8) c | 88.8 (±4.1) d |
| **Dicamba + 2,4-D** | | 82.5 (±3.9) c | 85.5 (±4.0) d |
| **Control** | **TF** | 70.8 (±3.6). c | 74.8 (±3.7) cd |
| **2,4-D** | | 74.3 (±3.7) c | 75.2 (±3.8) cd |

| Continued | | | |
|---|---|---|---|
| **Dicamba** | **TF** | 68.7 (±3.6) c | 77.3 (±3.8) cd |
| **Aminopyralid** | | 41.3 (±2.8) ab | 41.3 (±2.8) ab |
| **2,4-D + Clopyralid** | | 73.8 (±3.7) c | 81.2 (±3.9) d |
| **Dicamba + 2,4-D** | | 72.2 (±3.7) c | 76.8 (±3.8) cd |

Table B-27. Viability of seeds of four different Italian ryegrass biotypes after synthetic auxin herbicide treatment applied at two growth stages in greenhouse 2018 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | **Anthesis** | **Boot** |
| **Treatments** | **Biotype** | **Viability (SE)** | |
| | | ---------- % ---------- | |
| **Control** | **FG** | 93.2 (±2.8) gh | 93.2 (±2.8) h |
| **2,4-D** | | 92.8 (±2.8) gh | 93.8 (±2.8) h |
| **Dicamba** | | 96.4 (±2.8) gh | 89.4 (±2.7) h |
| **Aminopyralid** | | 26.0 (±1.5) a | 42.6 (±1.9) abc |
| **2,4-D + Clopyralid** | | 94.0 (±2.8) gh | 94.2 (±2.8) h |
| **Dicamba + 2,4-D** | | 94.2 (±2.8) gh | 95.6 (±2.8) h |
| **2,4-D (2X)** | | 95.8 (±2.8) gh | 91.8 (±2.8) h |
| **Dicamba (2X)** | | 92.2 (±2.8) gh | 88.6 (±2.7) h |
| **Halauxifen-methyl** | | 94.4 (±2.8) gh | 94.2 (±2.8) h |
| **Control** | **PR** | 95.0 (±2.8) gh | 95.0 (±2.8) h |
| **2,4-D** | | 94.0 (±2.8) gh | 87.6 (±2.7) h |
| **Dicamba** | | 92.4 (±2.8) gh | 81.0 (±2.6) gh |
| **Aminopyralid** | | 23.6 (±1.4) a | 47.8 (±2.0) bcd |
| **2,4-D + Clopyralid** | | 93.4 (±2.8) gh | 94.4 (±2.8). h |
| **Dicamba + 2,4-D** | | 89.8 (±2.7) fgh | 79.4 (±2.6) gh |
| **2,4-D (2X)** | | 90.6 (±2.8) gh | 91.0 (±2.8) h |
| **Dicamba (2X)** | | 87.2 (±2.7) fgh | 94.0 (±2.8) h |
| **Halauxifen-methyl** | | 85.2 (±2.7) fgh | 87.8 (±2.7) h |
| **Control** | **RD** | 92.8 (±2.8) gh | 93.4 (±2.8). h |
| **2,4-D** | | 90.0 (±2.8) fgh | 91.6 (±2.8). h |
| **Dicamba** | | 97.4 (±2.9) gh | 95.8 (±2.8) h |
| **Aminopyralid** | | 27.6 (±1.5) ab | 61.2 (±2.3) def |
| **2,4-D + Clopyralid** | | 92.0 (±2.8) gh | 92.8 (±2.8) h |
| **Dicamba + 2,4-D** | | 97.6 (±2.9) gh | 97.4 (±2.9) h |
| **2,4-D (2X)** | | 91.2 (±2.8) gh | 93.2 (±2.8) h |
| **Dicamba (2X)** | | 95.0 (±2.8) gh | 97.4 (±2.9) h |

| Continued | | | |
|---|---|---|---|
| **Halauxifen-methyl** | **RD** | 97.4 (±2.9) gh | 96.2 (±2.8) h |
| **Control** | TF | 87.8 (±2.7) fgh | 92.2 (±2.8) h |
| **2,4-D** | | 89.8 (±2.7) fgh | 90.0 (±2.8) h |
| **Dicamba** | | 92.2 (±2.8) gh | 91.4 (±2.8) h |
| **Aminopyralid** | | 49.8 (±2.0) cde | 68.4 (±2.4) efg |
| **2,4-D + Clopyralid** | | 88.2 (±2.7) fgh | 89.2 (±2.7) h |
| **Dicamba + 2,4-D** | | 87.2 (±2.7) fgh | 92.6 (±2.8) h |
| **2,4-D (2X)** | | 87.8 (±2.7) fgh | 91.0 (±2.8) h |
| **Dicamba (2X)** | | 82.6 (±2.6) fgh | 84.2 (±2.7) h |
| **Halauxifen-methyl** | | 91.6 (±2.8) gh | 86.0 (±2.7) h |

Table B-28. Seed weight of four different Italian ryegrass biotypes after synthetic auxin herbicide treatment applied at two growth stages in greenhouse 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | **Growth Stage** | |
|---|---|---|---|
| | | **Anthesis** | **Boot** |
| **Treatment** | **Biotype** | **Seed Weight (SE)** | |
| | | ---------- g/1,000 seeds ---------- | |
| **Control** | **FG** | 2.145 (±0.127) c | 2.185 (±0.128). c |
| **2,4-D** | | 2.030 (±0.123) bc | 2.195 (±0.129). c |
| **Dicamba** | | 2.070 (±0.125) bc | 2.343 (±0.133) c |
| **Aminopyralid** | | 1.204 (±0.094). a | 1.300 (±0.098) ab |
| **2,4-D + Clopyralid** | | 2.171 (±0.128) c | 2.375 (±0.134) c |
| **Dicamba + 2,4-D** | | 2.197 (±0.129) c | 2.262 (±0.131) c |
| **Control** | **PR** | 2.663 (±0.142) b | 2.143 (±0.127) b |
| **2,4-D** | | 2.225 (±0.129) b | 2.266 (±0.131) b |
| **Dicamba** | | 2.730 (±0.144) b | 2.303 (±0.132) b |
| **Aminopyralid** | | 1.244 (±0.096) a | 1.172 (±0.093) a |
| **2,4-D + Clopyralid** | | 2.224 (±0.129) b | 2.275 (±0.131) b |
| **Dicamba + 2,4-D** | | 2.668 (±0.142) b | 2.458 (±0.136) b |
| **Control** | **RD** | 2.137 (±0.127) d | 2.097 (±0.126). d |
| **2,4-D** | | 2.205 (±0.129) d | 2.029 (±0.123) d |
| **Dicamba** | | 2.277 (±0.131) d | 2.035 (±0.124) d |
| **Aminopyralid** | | 1.190 (±0.094) ab | 1.229 (±0.095) ac |
| **2,4-D + Clopyralid** | | 2.218 (±0.129) d | 1.859 (±0.118) bd |
| **Dicamba + 2,4-D** | | 1.856 (±0.118) cd | 2.191 (±0.128) d |
| **Control** | **TF** | 2.284 (±0.131) de | 2.159 (±0.127) de |
| **2,4-D** | | 2.291 (±0.131). de | 2.282 (±0.131) de |

| Continued | | | |
|---|---|---|---|
| **Dicamba** | **TF** | 2.145 (±0.127). de | 1.773 (±0.115) bd |
| **Aminopyralid** | | 1.216 (±0.095). ab | 1.234 (±0.096) ac |
| **2,4-D + Clopyralid** | | 1.984 (±0.122) cde | 2.414 (±0.135) e |
| **Dicamba + 2,4-D** | | 2.189 (±0.128) de | 2.220 (±0.129) de |

Table B-29. Seed weight of four different Italian ryegrass biotypes after synthetic auxin herbicide treatment applied at two growth stages in greenhouse 2018 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | **Growth Stage** | |
|---|---|---|---|
| | | **Anthesis** | **Boot** |
| **Treatments** | **Biotype** | **Seed Weight (SE)** | |
| | | ---------- g/1,000 seeds ---------- | |
| **Control** | **FG** | 2.305 (±0.158) c | 2.001 (±0.147) b |
| **2,4-D** | | 1.817 (±0.140) bc | 1.839 (±0.141) b |
| **Dicamba** | | 1.979 (±0.146) bc | 2.096 (±0.150) b |
| **Aminopyralid** | | 0.981 (±0.102) a | 1.043 (±0.105) a |
| **2,4-D + Clopyralid** | | 1.914 (±0.143) bc | 2.008 (±0.147) b |
| **Dicamba + 2,4-D** | | 1.729 (±0.136) bc | 1.957 (±0.145) b |
| **2,4-D (2X)** | | 1.652 (±0.133) b | 1.901 (±0.143) b |
| **Dicamba (2X)** | | 1.978 (±0.146) bc | 2.146 (±0.152) b |
| **Halauxifen-methyl** | | 2.063 (±0.149) bc | 2.059 (±0.149) b |
| **Control** | **PR** | 2.083 (±0.150) b | 2.028 (±0.148) b |
| **2,4-D** | | 2.186 (±0.154) b | 1.895 (±0.143) b |
| **Dicamba** | | 2.148 (±0.152) b | 2.064 (±0.149) b |
| **Aminopyralid** | | 1.113 (±0.108) a | 1.122 (±0.109) a |
| **2,4-D + Clopyralid** | | 1.846 (±0.141) b | 1.784 (±0.138) b |
| **Dicamba + 2,4-D** | | 1.946 (±0.145) b | 2.243 (±0.156) b |
| **2,4-D (2X)** | | 1.897 (±0.143) b | 2.074 (±0.150) b |
| **Dicamba (2X)** | | 2.159 (±0.153) b | 2.104 (±0.151) b |
| **Halauxifen-methyl** | | 2.141 (±0.152) b | 2.199 (±0.154) b |
| **Control** | **RD** | 2.320 (±0.159) b | 2.106 (±0.151) b |
| **2,4-D** | | 1.852 (±0.141) b | 2.125 (±0.151) b |
| **Dicamba** | | 2.076 (±0.150) b | 2.095 (±0.150) b |
| **Aminopyralid** | | 1.074 (±0.107) a | 1.064 (±0.106) a |
| **2,4-D + Clopyralid** | | 2.082 (±0.150) b | 1.791 (±0.139) b |
| **Dicamba + 2,4-D** | | 1.908 (±0.143) b | 1.710 (±0.135) b |
| **2,4-D (2X)** | | 1.929 (±0.144) b | 1.792 (±0.139) b |
| **Dicamba (2X)** | | 1.934 (±0.144) b | 1.673 (±0.134) b |

| Continued | | | |
|---|---|---|---|
| **Halauxifen-methyl** | **RD** | 1.932 (±0.144) b | 2.002 (±0.147) b |
| **Control** | **TF** | 2.128 (±0.152) b | 2.007 (±0.147) b |
| **2,4-D** | | 1.884 (±0.142) b | 1.946 (±0.145) b |
| **Dicamba** | | 1.937 (±0.144) b | 2.022 (±0.148) b |
| **Aminopyralid** | | 0.952 (±0.100) a | 1.038 (±0.105) a |
| **2,4-D + Clopyralid** | | 2.148 (±0.152) b | 2.150 (±0.152) b |
| **Dicamba + 2,4-D** | | 1.893 (±0.143) b | 2.108 (±0.151) b |
| **2,4-D (2X)** | | 1.923 (±0.144) b | 1.664 (±0.133) b |
| **Dicamba (2X)** | | 1.995 (±0.147) b | 1.939 (±0.144) b |
| **Halauxifen-methyl** | | 1.970 (±0.146) b | 1.967 (±0.146) b |

Table B-30. Seed viability reduction after accelerating aging test of four different Italian ryegrass biotypes after synthetic auxin herbicide treatment applied at two growth stages in greenhouse 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | **Growth Stage** | |
|---|---|---|---|
| | | **Anthesis** | **Boot** |
| **Treatment** | **Biotype** | **Viability Reduction (SE)** | |
| | | ---------- % ---------- | |
| **Control** | **FG** | 2.500 (±0.618) abc | 6.167 (±2.170) ab |
| **2,4-D** | | 5.667 (±2.080) abc | 3.000 (±1.513) ab |
| **Dicamba** | | 5.000 (±1.954) abc | 3.000 (±1.513) ab |
| **Aminopyralid** | | 6.833 (±2.284) abc | 0.000 (±0.000) abc |
| **2,4-D + Clopyralid** | | 11.000 (±2.898) abc | 12.167 (±3.048) abc |
| **Dicamba + 2,4-D** | | 0.333 (±0.504) abc | 4.500 (±1.854) ab |
| **Control** | **PR** | 2.333 (±1.335) abc | 1.667 (±1.128) ab |
| **2,4-D** | | 5.833 (±2.110) abc | 2.000 (±1.236) ab |
| **Dicamba** | | 8.500 (±2.547) abc | 6.833 (±2.284) ab |
| **Aminopyralid** | | 4.833 (±1.921) abc | 6.000 (±2.14) ab |
| **2,4-D + Clopyralid** | | 4.167 (±1.784) abc | 4.000 (±1.748) ab |
| **Dicamba + 2,4-D** | | 3.667 (±1.673) abc | 3.000 (±1.513) ab |
| **Control** | **RD** | 10.667 (±2.854) abc | 9.000 (±2.621) abc |
| **2,4-D** | | 9.333 (±2.669) abc | 9.333 (±2.669) abc |
| **Dicamba** | | 6.667 (±2.256) abc | 10.333 (±2.809) abc |
| **Aminopyralid** | | 1.500 (±1.070) abc | 12.000 (±3.027) abc |
| **2,4-D + Clopyralid** | | 10.667 (±2.854) abc | 5.167 (±1.986) ab |
| **Dicamba + 2,4-D** | | 5.167 (±1.986) abc | 4.667 (±1.888) ab |

| Continued | | | |
|---|---|---|---|
| **Control** | **TF** | 8.000 (±2.471) abc | 10.167 (±2.786) ab |
| **2,4-D** | | 25.833 (±4.441) abc | 29.000 (±4.705) bc |
| **Dicamba** | | 12.333 (±3.069) abc | 23.833 (±4.266) abc |
| **Aminopyralid** | | 16.167 (±3.513) abc | 5.167 (±1.986) a |
| **2,4-D + Clopyralid** | | 24.000 (±4.281) abc | 36.000 (±5.243) c |
| **Dicamba + 2,4-D** | | 12.833 (±3.130) abc | 22.833 (±4.175) abc |

Table B-31. Seed viability reduction after accelerating aging test of four different Italian ryegrass biotypes after synthetic auxin herbicide treatment applied at two growth stages in greenhouse 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | **Growth Stage** | |
|---|---|---|---|
| | | **Anthesis** | **Boot** |
| **Treatments** | **Biotype** | **Viability Reduction (SE)** | |
| | | ---------- % ---------- | |
| **Control** | **FG** | 9.196 (±2.140) abcde | 9.196 (±2.140) abcde |
| **2,4-D** | | 9.196 (±2.140) abcde | 8.196 (±2.016) abc |
| **Dicamba** | | 7.597 (±1.939) abc | 8.796 (±2.091) abcd |
| **Aminopyralid** | | 0.600 (±0.538) abcde | 3.998 (±1.397) ab |
| **2,4-D + Clopyralid** | | 9.596 (±2.188) abcde | 6.397 (±1.775) abc |
| **Dicamba + 2,4-D** | | 7.997 (±1.991) abcd | 8.396 (±2.042) abc |
| **2,4-D (2X)** | | 8.796 (±2.091) abcde | 6.797 (±1.831) abc |
| **Dicamba (2X)** | | 6.997 (±1.859) abc | 7.597 (±1.939) abc |
| **Halauxifen-methyl** | | 8.197 (±2.017) abcd | 7.597 (±1.939) abc |
| **Control** | **PR** | 21.757 (±3.367) bcdefg | 21.735 (±3.364) cdef |
| **2,4-D** | | 8.583 (±2.064) abcd | 8.574 (±2.062) abcd |
| **Dicamba** | | 11.377 (±2.389) abcdef | 8.774 (±2.086) abcd |
| **Aminopyralid** | | 2.794 (±1.164) a | 10.369 (±2.275) abcde |
| **2,4-D + Clopyralid** | | 10.779 (±2.322) abcde | 5.982 (±1.713) ab |
| **Dicamba + 2,4-D** | | 13.972 (±2.660) abcdefg | 17.149 (±2.963) abcdef |
| **2,4-D (2X)** | | 11.577 (±2.410) abcdef | 7.179 (±1.881). abc |
| **Dicamba (2X)** | | 13.972 (±2.660) abcdefg | 14.158 (±2.677) abcdef |
| **Halauxifen-methyl** | | 8.982 (±2.113) abcde | 14.158 (±2.677) abcdef |
| **Control** | **RD** | 2.798 (±1.166) a | 4.194 (±1.431) ab |
| **2,4-D** | | 9.592 (±2.187) abcde | 3.794 (±1.360) ab |
| **Dicamba** | | 5.795 (±1.687) abc | 8.188 (±2.014) abc |
| **Aminopyralid** | | 0.000 (±0.000) abcdefg | 1.598 (±0.879) a |
| **2,4-D + Clopyralid** | | 2.798 (±1.166) a | 4.393 (±1.465) ab |
| Continued | | | |

| | | | |
|---|---|---|---|
| **Dicamba + 2,4-D** | **RD** | 6.195 (±1.746) abc | 6.790 (±1.829) abc |
| **2,4-D (2X)** | | 8.193 (±2.016) abcd | 2.995 (±1.206) a |
| **Dicamba (2X)** | | 6.994 (±1.858) abc | 5.392 (±1.626) abc |
| **Halauxifen-methyl** | | 4.996 (±1.564) ab | 7.788 (±1.963) abc |
| **Control** | **TF** | 22.777 (±3.453) cdefg | 28.192 (±3.880) def |
| **2,4-D** | | 18.182 (±3.059) abcdefg | 19.795 (±3.203) bcdef |
| **Dicamba** | | 27.372 (±3.817). defg | 13.596 (±2.624) abcde |
| **Aminopyralid** | | 16.583 (±2.913) abcdefg | 34.391 (±4.332) f |
| **2,4-D + Clopyralid** | | 28.371 (±3.893) efg | 19.795 (±3.203) bcdef |
| **Dicamba + 2,4-D** | | 32.167 (±4.173) fg | 18.195 (±3.061) abcdef |
| **2,4-D (2X)** | | 34.765 (±4.357) g | 28.792 (±3.925) ef |
| **Dicamba (2X)** | | 20.180 (±3.235) bcdefg | 17.995 (±3.043) abcdef |
| **Halauxifen-methyl** | | 23.177 (±3.486) cdefg | 15.596 (±2.821) abcdef |

Table B-32. Seed viability test of four different Italian ryegrass biotypes after synthetic auxin herbicide treatment applied at two growth stages in greenhouse pulled data from both years (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | **Growth Stage** | |
|---|---|---|---|
| | | **Anthesis** | **Boot** |
| **Treatment** | **Biotype** | **Viability (SE)** | |
| | | ---------- % ---------- | |
| **Control** | **FG** | 82.2 (±6.0) e | 83.3 (±6.1) e |
| **2,4-D** | | 85.2 (±6.2) e | 83.4 (±6.1) e |
| **Dicamba** | | 84.5 (±6.2) e | 81.3 (±6.0) e |
| **Aminopyralid** | | 28.4 (±2.5) a | 37.5 (±3.1) ab |
| **2,4-D + Clopyralid** | | 85.4 (±6.2) e | 83.0 (±6.1) e |
| **Dicamba + 2,4-D** | | 79.9 (±5.9) de | 82.4 (±6.0) e |
| **Control** | **PR** | 85.0 (±6.2) e | 89.5 (±6.5) e |
| **2,4-D** | | 82.8 (±6.1) e | 83.3 (±6.1) e |
| **Dicamba** | | 83.1 (±6.1) e | 74.7 (±5.5) de |
| **Aminopyralid** | | 33.8 (±2.9) ab | 47.3 (±3.8) bc |
| **2,4-D + Clopyralid** | | 82.2 (±6.0) e | 85.3 (±6.2) e |
| **Dicamba + 2,4-D** | | 81.3 (±6.0) de | 79.8 (±5.9) e |
| **Control** | **RD** | 88.3 (±6.4) e | 86.3 (±6.3) e |
| **2,4-D** | | 83.4 (±6.1) e | 84.0 (±6.1) e |
| **Dicamba** | | 92.0 (±6.6) e | 87.2 (±6.3) e |
| **Aminopyralid** | | 37.2 (±3.1) ab | 60.6 (±4.6) cd |

| Continued | | | |
|---|---|---|---|
| **2,4-D + Clopyralid** | **RD** | 83.4 (±6.1) e | 90.5 (±6.6) e |
| **Dicamba + 2,4-D** | | 88.6 (±6.4) e | 91.7 (±6.6) e |
| **Control** | **TF** | 78.7 (±5.8) de | 83.6 (±6.1) e |
| **2,4-D** | | 81.3 (±6.0) de | 81.4 (±6.0) e |
| **Dicamba** | | 81.4 (±6.0) de | 84.1 (±6.1) e |
| **Aminopyralid** | | 45.5 (±3.7) bc | 54.7 (±4.2) c |
| **2,4-D + Clopyralid** | | 81.7 (±6.0) de | 84.3 (±6.2) e |
| **Dicamba + 2,4-D** | | 79.8 (±5.9) de | 83.0 (±6.1) e |

Table B-33. Seed viability reduction after aging test of four different Italian ryegrass biotypes after synthetic auxin herbicide treatment applied at two growth stages in greenhouse pulled data from both years (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatment | Biotype | Viability Reduction | |
| | | % | |
| **Control** | **FG** | 4.912 (±1.575) acd | 7.302 (±1.923) abce |
| **2,4-D** | | 8.012 (±2.013) abcdef | 5.904 (±1.728) abce |
| **Dicamba** | | 5.900 (±1.728) abcdef | 6.204 (±1.772) abce |
| **Aminopyralid** | | 4.300 (±1.476) acd | 2.103 (±1.006) ab |
| **2,4-D + Clopyralid** | | 10.201 (±2.273) abcdef | 9.912 (±2.239) abcdef |
| **Dicamba + 2,4-D** | | 4.201 (±1.458) acd | 5.800 (±1.714) abce |
| **Control** | **PR** | 12.100 (±2.475) abcdef | 11.900 (±2.455) abcdef |
| **2,4-D** | | 7.400 (±1.936) abcdef | 5.300 (±1.638) abce |
| **Dicamba** | | 10.600 (±2.317) abcdef | 8.300 (±2.050) abcdef |
| **Aminopyralid** | | 4.300 (±1.476) acd | 8.400 (±2.062) abcdef |
| **2,4-D + Clopyralid** | | 7.00 (±1.883) abcdef | 5.100 (±1.607) abce |
| **Dicamba + 2,4-D** | | 8.400 (±2.062) abcdef | 10.400 (±2.295) abcdef |
| **Control** | **RD** | 7.400 (±1.936) abcdef | 6.500 (±1.814) abce |
| **2,4-D** | | 9.200 (±2.158) abcdef | 6.100 (±1.757) abce |
| **Dicamba** | | 6.400 (±1.800) abcdef | 9.300 (±2.170) abcdef |
| **Aminopyralid** | | 0.800 (±0.636) abcdef | 7.600 (±1.962) abce |
| **2,4-D + Clopyralid** | | 6.200 (±1.772) abcdef | 5.013 (±1.591) abce |
| **Dicamba + 2,4-D** | | 6.002 (±1.743) abcdef | 5.302 (±1.638) abce |
| **Control** | **TF** | 15.101 (±2.765) abcdef | 19.300 (±3.126) abcdef |
| **2,4-D** | | 20.200 (±3.198) abcdef | 23.000 (±3.413) cdef |
| **Dicamba** | | 21.100 (±3.269) abcdef | 21.100 (±3.269) abcdef |
| **Aminopyralid** | | 17.012 (±2.934) abcdef | 19.900 (±3.174) abcdef |
| **2,4-D + Clopyralid** | | 25.300 (±3.579) bef | 28.300 (±3.785) df |
| **Dicamba + 2,4-D** | | 22.700 (±3.390) abcdef | 19.600 (±3.150) abcdef |

Table B-34. Parameters and standard errors (SE) of fitted log-logistic model for speed of germination on anthesis growth stage in greenhouse trial 2017.

| | | Parameter | | | | |
|---|---|---|---|---|---|---|
| **Biotype** | **Treatment** | **Slope (SE)** | **MaxGerm (SE)** | **MaxGerm Ratio** | **T50 (SE)** | **T50 Ratio** |
| **FG** | **Control** | -5.132 (±0.361) | 0.845 (±0.038) | 1.000 | 10.018 (±0.276) | 1.000 |
| | **2,4-D** | -4.432 (±0.304) | 0.900 (±0.043) | 1.065 | 9.904 (±0.328) | 0.989 |
| | **Dicamba** | -4.397 (±0.355) | 0.880 (±0.063) | 1.041 | 10.773 (±0.472) | 1.075 |
| | **Aminopyralid** | -3.097 (±0.414) | 0.359 (±0.063) | 0.425 | 11.245 (±1.438) | 1.122 |
| | **2,4-D + Clopyralid** | -4.438 (±0.347) | 0.932 (±0.063) | 1.103 | 10.746 (±0.448) | 1.073 |
| | **Dicamba + 2,4-D** | -4.224 (±0.364) | 0.825 (±0.068) | 0.976 | 10.963 (±0.558) | 1.094 |
| **PR** | **Control** | -6.757 (±0.333) | 0.756 (±0.018) | 1.000 | 6.853 (±0.091) | 1.000 |
| | **2,4-D** | -4.661 (±0.277) | 0.790 (±0.025) | 1.045 | 8.478 (±0.205) | 1.237 |
| | **Dicamba** | -5.043 (±0.276) | 0.735 (±0.021) | 0.972 | 7.472 (±0.147) | 1.090 |
| | **Aminopyralid** | -4.964 (±0.371) | 0.463 (±0.024) | 0.612 | 8.359 (±0.236) | 1.220 |
| | **2,4-D + Clopyralid** | -4.794 (±0.295) | 0.746 (±0.026) | 0.987 | 8.646 (±0.211) | 1.262 |
| | **Dicamba + 2,4-D** | -4.881 (±0.28) | 0.803 (±0.023) | 1.062 | 8.411 (±0.186) | 1.227 |
| **RD** | **Control** | -4.911 (±0.225) | 0.910 (±0.013) | 1.000 | 6.204 (±0.103) | 1.000 |
| | **2,4-D** | -8.317 (±0.399) | 0.729 (±0.018) | 0.801 | 6.311 (±0.070) | 1.017 |
| | **Dicamba** | -8.120 (±0.367) | 0.852 (±0.015) | 0.936 | 6.510 (±0.068) | 1.049 |
| | **Aminopyralid** | -9.276 (±0.546) | 0.512 (±0.020) | 0.563 | 6.460 (±0.078) | 1.041 |
| | **2,4-D + Clopyralid** | -4.685 (±0.267) | 0.751 (±0.022) | 0.825 | 7.812 (±0.175) | 1.259 |
| | **Dicamba + 2,4-D** | -7.75 (±0.360) | 0.812 (±0.016) | 0.892 | 6.560 (±0.073) | 1.057 |
| **TF** | **Control** | -6.196 (±0.375) | 0.477 (±0.020) | 1.000 | 5.896 (±0.105) | 1.000 |
| | **2,4-D** | -4.940 (±0.294) | 0.562 (±0.021) | 1.178 | 6.626 (±0.142) | 1.124 |
| | **Dicamba** | -4.808 (±0.320) | 0.420 (±0.021) | 0.881 | 5.884 (±0.146) | 0.998 |
| | **Aminopyralid** | -4.918 (±0.364) | 0.438 (±0.022) | 0.918 | 7.904 (±0.219) | 1.341 |
| | **2,4-D + Clopyralid** | -3.980 (±0.265) | 0.642 (±0.027) | 1.346 | 8.044 (±0.257) | 1.364 |
| | **Dicamba + 2,4-D** | -4.124 (±0.123) | 0.600 (±0.031) | 1.258 | 7.606 (±0.221) | 1.290 |

Table B-35. Parameters and standard errors (SE) of fitted log-logistic model for speed of germination on boot growth stage in greenhouse trial 2017.

| | | Parameter | | | | |
|---|---|---|---|---|---|---|
| **Biotype** | **Treatment** | **Slope (SE)** | **MaxGerm (SE)** | **MaxGerm Ratio** | **T50 (SE)** | **T50 Ratio** |
| **FG** | **Control** | -3.477 (±0.286) | 0.952 (±0.092) | 1.000 | 11.256 (±0.757) | 1.000 |
| | **2,4-D** | -5.438 (±0.421) | 0.846 (±0.043) | 0.889 | 10.441 (±0.301) | 0.079 |
| | **Dicamba** | -6.334 (±0.485) | 0.810 (±0.033) | 0.851 | 10.267 (±0.223) | 0.076 |
| | **Aminopyralid** | -2.725 (±0.384) | 0.343 (±0.073) | 0.360 | 11.743 (±1.988) | 0.032 |
| | **2,4-D + Clopyralid** | -4.260 (±0.350) | 0.920 (±0.072) | 0.966 | 11.013 (±0.533) | 0.086 |
| | **Dicamba + 2,4-D** | -3.355 (±0.301) | 0.990 (±0.128) | 1.040 | 12.109 (±1.049) | 0.092 |
| **PR** | **Control** | -6.095 (±0.292) | 0.850 (±0.016) | 1.000 | 7.214 (±0.103) | 0.139 |
| | **2,4-D** | -5.585 (±0.281) | 0.831 (±0.018) | 0.978 | 7.560 (±0.125) | 0.136 |
| | **Dicamba** | -4.560 (±0.283) | 0.758 (±0.027) | 0.892 | 8.616 (±0.225) | 0.124 |
| | **Aminopyralid** | -4.264 (±0.353) | 0.472 (±0.029) | 0.555 | 8.855 (±0.340) | 0.077 |
| | **2,4-D + Clopyralid** | -4.800 (±0.287) | 0.818 (±0.026) | 0.962 | 8.802 (±0.211) | 0.133 |
| | **Dicamba + 2,4-D** | -5.521 (±0.276) | 0.843 (±0.017) | 0.992 | 7.528 (±0.125) | 0.137 |
| **RD** | **Control** | -5.000 (±0.273) | 0.795 (±0.021) | 1.000 | 7.897 (±0.159) | 0.127 |
| | **2,4-D** | -6.740 (±0.334) | 0.728 (±0.018) | 0.916 | 6.630 (±0.089) | 0.116 |
| | **Dicamba** | -5.403 (±0.279) | 0.766 (±0.019) | 0.964 | 7.207 (±0.125) | 0.122 |
| | **Aminopyralid** | -12.410 (±0.710) | 0.542 (±0.020) | 0.682 | 6.212 (±0.064) | 0.086 |
| | **2,4-D + Clopyralid** | -6.246 (±0.289) | 0.889 (±0.014) | 1.118 | 7.063 (±0.095) | 0.142 |
| | **Dicamba + 2,4-D** | -5.552 (±0.273) | 0.871 (±0.016) | 1.096 | 7.575 (±0.123) | 0.139 |
| **TF** | **Control** | -6.496 (±0.394) | 0.476 (±0.020) | 1.000 | 5.685 (±0.097) | 0.176 |
| | **2,4-D** | -4.074 (±0.267) | 0.522 (±0.022) | 1.097 | 6.753 (±0.196) | 0.193 |
| | **Dicamba** | -4.810 (±0.278) | 0.638 (±0.021) | 1.340 | 7.053 (±0.152) | 0.236 |
| | **Aminopyralid** | -4.881 (±0.390) | 0.450 (±0.025) | 0.945 | 8.784 (±0.275) | 0.166 |
| | **2,4-D + Clopyralid** | -3.325 (±0.240) | 0.685 (±0.037) | 1.439 | 8.615 (±0.399) | 0.253 |
| | **Dicamba + 2,4-D** | -4.119 (±0.266) | 0.571 (±0.023) | 1.200 | 7.178 (±0.205) | 0.211 |

Table B-36. Parameters and standard errors (SE) of fitted log-logistic model for speed of germination on anthesis growth stage in greenhouse trial 2018.

| | | Parameter | | | | |
|---|---|---|---|---|---|---|
| **Biotype** | **Treatment** | **Slope (SE)** | **MaxGerm (SE)** | **MaxGerm Ratio** | **T50 (SE)** | **T50 Ratio** |
| **FG** | **Control** | -6.245 (±0.308) | 0.902 (±0.013) | 1.000 | 5.038 (±0.072) | 1.000 |
| | **2,4-D** | -5.613 (±0.273) | 0.899 (±0.014) | 0.997 | 5.197 (±0.082) | 1.032 |
| | **2,4-D (2X)** | -5.544 (±0.264) | 0.934 (±0.012) | 1.035 | 5.320 (±0.083) | 1.056 |
| | **2,4-D + Clopyralid** | -6.766 (±0.341) | 0.901 (±0.013) | 0.999 | 5.059 (±0.067) | 1.004 |
| | **Aminopyralid** | -5.526 (±0.351) | 0.535 (±0.023) | 0.593 | 5.946 (±0.123) | 1.180 |
| | **Dicamba** | -7.843 (±0.416) | 0.944 (±0.010) | 1.047 | 4.896 (±0.056) | 0.972 |
| | **Dicamba (2X)** | -8.431 (±0.465) | 0.912 (±0.013) | 1.011 | 5.083 (±0.054) | 1.009 |
| | **Dicamba + 2,4-D** | -8.039 (±0.438) | 0.922 (±0.012) | 1.022 | 4.898 (±0.056) | 0.972 |
| | **Halauxifen-methyl** | -5.882 (±0.283) | 0.925 (±0.012) | 1.025 | 5.225 (±0.077) | 1.037 |
| **PR** | **Control** | -7.075 (±0.364) | 0.793 (±0.018) | 1.000 | 5.638 (±0.076) | 1.000 |
| | **2,4-D** | -6.004 (±0.288) | 0.920 (±0.013) | 1.160 | 6.052 (±0.088) | 1.073 |
| | **2,4-D (2X)** | -7.811 (±0.393) | 0.845 (±0.016) | 1.066 | 5.593 (±0.067) | 0.992 |
| | **2,4-D + Clopyralid** | -7.271 (±0.346) | 0.888 (±0.014) | 1.120 | 5.997 (±0.074) | 1.064 |
| | **Aminopyralid** | -6.506 (±0.511) | 0.336 (±0.021) | 0.424 | 6.128 (±0.137) | 1.087 |
| | **Dicamba** | -5.883 (±0.290) | 0.877 (±0.015) | 1.106 | 6.092 (±0.093) | 1.081 |
| | **Dicamba (2X)** | -5.407 (±0.290) | 0.807 (±0.019) | 1.018 | 6.719 (±0.119) | 1.192 |
| | **Dicamba + 2,4-D** | -5.824 (±0.296) | 0.824 (±0.017) | 1.039 | 5.983 (±0.095) | 1.061 |
| | **Halauxifen-methyl** | -5.327 (±0.299) | 0.691 (±0.021) | 0.871 | 5.936 (±0.112) | 1.053 |
| **RD** | **Control** | -6.954 (±0.334) | 0.918 (±0.012) | 1.000 | 5.626 (±0.071) | 1.000 |
| | **2,4-D** | -7.807 (±0.378) | 0.847 (±0.016) | 0.923 | 6.037 (±0.072) | 1.071 |
| | **2,4-D (2X)** | -7.552 (±0.368) | 0.853 (±0.016) | 0.929 | 5.819 (±0.071) | 1.032 |
| | **2,4-D + Clopyralid** | -6.791 (±0.322) | 0.915 (±0.013) | 0.997 | 5.909 (±0.077) | 1.048 |
| | **Aminopyralid** | -6.726 (±0.450) | 0.459 (±0.022) | 0.500 | 5.866 (±0.108) | 1.040 |
| | **Dicamba** | -5.885 (±0.276) | 0.965 (±0.009) | 1.051 | 5.789 (±0.084) | 1.027 |
| | **Dicamba (2X)** | -6.736 (±0.316) | 0.936 (±0.011) | 1.020 | 6.254 (±0.081) | 1.109 |

| | | | Continued | | | |
|---|---|---|---|---|---|---|
| **RD** | **Dicamba + 2,4-D** | -8.902 (±0.406) | 0.966 (±0.008) | 1.052 | 5.780 (±0.059) | 1.025 |
| | **Halauxifen-methyl** | -9.623 (±0.439) | 0.944 (±0.010) | 1.028 | 5.836 (±0.057) | 1.035 |
| **TF** | **Control** | -6.176 (±0.335) | 0.715 (±0.020) | 1.000 | 5.820 (±0.093) | 1.000 |
| | **2,4-D** | -7.301 (±0.395) | 0.797 (±0.018) | 1.115 | 5.243 (±0.068) | 0.901 |
| | **2,4-D (2X)** | -5.730 (±0.338) | 0.613 (±0.022) | 0.857 | 5.400 (±0.100) | 0.928 |
| | **2,4-D + Clopyralid** | -8.232 (±0.493) | 0.616 (±0.022) | 0.862 | 5.518 (±0.074) | 0.948 |
| | **Aminopyralid** | -5.979 (±0.391) | 0.494 (±0.022) | 0.691 | 5.734 (±0.114) | 0.985 |
| | **Dicamba** | -5.646 (±0.309) | 0.710 (±0.021) | 0.993 | 5.646 (±0.099) | 0.970 |
| | **Dicamba (2X)** | -6.376 (±0.414) | 0.507 (±0.022) | 0.709 | 5.488 (±0.101) | 0.943 |
| | **Dicamba + 2,4-D** | -5.214 (±0.305) | 0.641 (±0.022) | 0.897 | 5.944 (±0.120) | 1.021 |
| | **Halauxifen-methyl** | -6.238 (±0.332) | 0.747 (±0.020) | 1.045 | 5.669 (±0.088) | 0.974 |

Table B-37. Parameters and standard errors (SE) of fitted log-logistic model for speed of germination on boot growth stage in greenhouse trial 2018.

| | | Parameter | | | | |
|---|---|---|---|---|---|---|
| **Biotype** | **Treatment** | **Slope (SE)** | **MaxGerm (SE)** | **MaxGerm Ratio** | **T50 (SE)** | **T50 Ratio** |
| **FG** | **Control** | -6.244 (±0.308) | 0.902 (±0.013) | 1.000 | 5.038 (±0.072) | 1.000 |
| | **2,4-D** | -6.650 (±0.333) | 0.887 (±0.014) | 0.983 | 5.244 (±0.070) | 1.041 |
| | **2,4-D (2X)** | -7.874 (±0.390) | 0.911 (±0.013) | 1.010 | 5.462 (±0.062) | 1.084 |
| | **2,4-D + Clopyralid** | -8.228 (±0.453) | 0.938 (±0.011) | 1.040 | 4.844 (±0.054) | 0.961 |
| | **Aminopyralid** | -21.919 (±2.353) | 0.312 (±0.021) | 0.346 | 6.091 (±0.104) | 1.209 |
| | **Dicamba** | -6.117 (±0.304) | 0.869 (±0.015) | 0.963 | 5.42 (±0.0790) | 1.076 |
| | **Dicamba (2X)** | -5.177 (±0.255) | 0.876 (±0.015) | 0.971 | 5.348 (±0.092) | 1.062 |
| | **Dicamba + 2,4-D** | -8.050 (±0.408) | 0.932 (±0.011) | 1.033 | 5.324 (±0.059) | 1.057 |
| | **Halauxifen-methyl** | -7.558 (±0.395) | 0.922 (±0.012) | 1.022 | 5.025 (±0.059) | 0.997 |
| **PR** | **Control** | -7.075 (±0.364) | 0.793 (±0.018) | 1.000 | 5.638 (±0.076) | 1.000 |
| | **2,4-D** | -6.822 (±0.335) | 0.847 (±0.016) | 1.068 | 6.116 (±0.082) | 1.085 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | **Continued** | | | |
| | **2,4-D (2X)** | -5.860 (±0.288) | 0.908 (±0.014) | 1.145 | 6.443 (±0.098) | 1.143 |
| | **2,4-D + Clopyralid** | -6.210 (±0.303) | 0.914 (±0.013) | 1.153 | 5.204 (±0.073) | 0.923 |
| | **Aminopyralid** | -4.171 (±0.319) | 0.449 (±0.024) | 0.566 | 6.664 (±0.219) | 1.182 |
| **PR** | **Dicamba** | -5.693 (±0.309) | 0.726 (±0.020) | 0.916 | 5.962 (±0.103) | 1.057 |
| | **Dicamba (2X)** | -4.882 (±0.258) | 0.821 (±0.018) | 1.035 | 6.199 (±0.120) | 1.100 |
| | **Dicamba + 2,4-D** | -7.808 (±0.425) | 0.679 (±0.021) | 0.856 | 6.281 (±0.084) | 1.114 |
| | **Halauxifen-methyl** | -5.935 (±0.306) | 0.802 (±0.018) | 1.011 | 6.162 (±0.097) | 1.093 |
| | **Control** | -7.069 (±0.335) | 0.924 (±0.012) | 1.000 | 5.731 (±0.071) | 1.000 |
| | **2,4-D** | -8.215 (±0.387) | 0.901 (±0.013) | 0.975 | 5.849 (±0.066) | 1.021 |
| | **2,4-D (2X)** | -6.965 (±0.327) | 0.925 (±0.012) | 1.001 | 6.174 (±0.078) | 1.077 |
| | **2,4-D + Clopyralid** | -8.739 (±0.424) | 0.896 (±0.014) | 0.970 | 5.643 (±0.060) | 0.985 |
| **RD** | **Aminopyralid** | -5.151 (±0.296) | 0.752 (±0.021) | 0.814 | 7.076 (±0.142) | 1.235 |
| | **Dicamba** | -7.453 (±0.346) | 0.932 (±0.011) | 1.009 | 5.916 (±0.070) | 1.032 |
| | **Dicamba (2X)** | -8.026 (±0.365) | 0.970 (±0.008) | 1.050 | 6.267 (±0.068) | 1.094 |
| | **Dicamba + 2,4-D** | -8.501 (±0.391) | 0.963 (±0.009) | 1.042 | 5.742 (±0.061) | 1.002 |
| | **Halauxifen-methyl** | -7.800 (±0.366) | 0.936 (±0.011) | 1.013 | 6.433 (±0.073) | 1.122 |
| | **Control** | -6.176 (±0.335) | 0.715 (±0.020) | 1.000 | 5.819 (±0.093) | 1.000 |
| | **2,4-D** | -7.081 (±0.375) | 0.733 (±0.020) | 1.025 | 5.802 (±0.081) | 0.997 |
| | **2,4-D (2X)** | -6.212 (±0.337) | 0.710 (±0.021) | 0.993 | 5.998 (±0.096) | 1.031 |
| | **2,4-D + Clopyralid** | -5.363 (±0.289) | 0.763 (±0.020) | 1.067 | 6.190 (±0.111) | 1.064 |
| **TF** | **Aminopyralid** | -6.706 (±0.413) | 0.549 (±0.022) | 0.768 | 5.756 (±0.097) | 0.989 |
| | **Dicamba** | -6.447 (±0.321) | 0.841 (±0.017) | 1.176 | 5.801 (±0.082) | 0.997 |
| | **Dicamba (2X)** | -4.725 (±0.266) | 0.739 (±0.021) | 1.034 | 6.297 (±0.134) | 1.082 |
| | **Dicamba + 2,4-D** | -5.687 (±0.294) | 0.807 (±0.018) | 1.129 | 6.106 (±0.100) | 1.049 |
| | **Halauxifen-methyl** | -5.877 (±0.318) | 0.725 (±0.020) | 1.014 | 5.947 (±0.099) | 1.022 |

Table B-38. Viability of seeds of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Dallas, OR 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatment | Species | Viability (SE) | |
| | | ---------- % ---------- | |
| **Control** | **Italian Ryegrass** | 82.75 (±1.06) a | 82.37 (±1.06) a |
| **2,4-D** | | 80.50 (±1.48) a | 82.00 (±1.50) a |
| **Dicamba** | | 81.75 (±1.49) a | 83.25 (±1.50) a |
| **2,4-D + Clopyralid** | | 84.00 (±1.51) a | 81.50 (±1.49) a |
| **Dicamba + 2,4-D** | | 84.75 (±1.51) a | 82.75 (±1.50) a |
| **Control** | **Tall Fescue** | 97.50 (±1.15) b | 97.50 (±1.15) b |
| **2,4-D** | | 98.50 (±1.63) b | 98.50 (±1.63) b |
| **Dicamba** | | 97.00 (±1.62) b | 97.00 (±1.62) b |
| **2,4-D + Clopyralid** | | 95.00 (±1.60) b | 95.00 (±1.60) b |
| **Dicamba + 2,4-D** | | 96.00 (±1.61) b | 96.00 (±1.61) b |

Table B-39. Hyslop viability of seeds of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Corvallis, OR Hyslop farm 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatment | Species | Viability (SE) | |
| | | ---------- % ---------- | |
| **Control** | **Italian Ryegrass** | 90.75 ( ±1.22) e | 90.75 ( ±1.22) e |
| **2,4-D** | | 90.25 ( ±1.22) e | 91.00 ( ±1.22) e |
| **Dicamba** | | 89.25 ( ±1.21) e | 90.00 ( ±1.21) e |
| **Aminopyralid** | | 30.50 ( ±0.66) c | 40.25 ( ±0.76) d |
| **2,4-D + Clopyralid** | | 90.25 ( ±1.22) e | 89.50 ( ±1.21) e |
| **Dicamba + 2,4-D** | | 91.75 ( ±1.23) e | 91.75 ( ±1.23) e |
| **Control** | **Tall Fescue** | 94.00 ( ±1.25) e | 93.00 ( ±1.24) e |
| **2,4-D** | | 92.25 ( ±1.23) e | 91.00 ( ±1.22) e |
| **Dicamba** | | 90.50 ( ±1.22) e | 89.25 ( ±1.21) e |
| **Aminopyralid** | | 11.50 ( ±0.39) a | 17.75 ( ±0.49) b |
| **2,4-D + Clopyralid** | | 88.75 ( ±1.20) e | 89.75 ( ±1.21) e |
| **Dicamba + 2,4-D** | | 91.00 ( ±1.22) e | 92.75 ( ±1.24) e |

Table B-40. Viability of seeds of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Gaston, OR 2018 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatments | Species | Viability (SE) | |
| | | ---------- % ---------- | |
| **Control** | **Italian Ryegrass** | 91.25 (±1.28) de | 93.00 (±1.29) de |
| **2,4-D** | | 92.00 (±1.28) de | 96.25 (±1.31) e |
| **Dicamba** | | 88.75 (±1.26) de | 91.75 (±1.28) de |
| **Aminopyralid** | | 42.00 (±0.86) b | 53.75 (±0.98) c |
| **2,4-D + Clopyralid** | | 91.25 (±1.28) de | 90.5 0(±1.27) de |
| **Dicamba + 2,4-D** | | 93.25 (±1.29) de | 92.25 (±1.28) de |
| **2,4-D (2X)** | | 89.75 (±1.27) de | 96.50 (±1.31) e |
| **Dicamba (2X)** | | 91.50 (±1.28) de | 89.00 (±1.26) d |
| **Halauxifen-methyl** | | 92.75 (±1.29) de | 94.25 (±1.30) de |
| **Control** | **Tall Fescue** | 96.75 (±1.32) de | 96.75 (±1.32) de |
| **2,4-D** | | 97.00 (±1.32) de | 94.50 (±1.30) de |
| **Dicamba** | | 94.75 (±1.30) de | 95.50 (±1.31) de |
| **Aminopyralid** | | 23.75 (±0.65). a | 48.50 ( ±0.93) bc |
| **2,4-D + Clopyralid** | | 97.00 (±1.32) de | 94.75 (±1.30) de |
| **Dicamba + 2,4-D** | | 93.25 (±1.29) de | 95.00 (±1.30) de |
| **2,4-D (2X)** | | 94.50 (±1.30) de | 94.75 (±1.30) de |
| **Dicamba (2X)** | | 94.75 (±1.30) de | 93.50 (±1.29) de |
| **Halauxifen-methyl** | | 97.50 (±1.32) de | 95.75 (±1.31) de |

Table B-41. Schmith viability of seeds of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Corvallis, OR Schmidt farm 2018 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatments | Species | Viability Reduction (SE) | |
| | | ---------- % ---------- | |
| **Control** | **Italian Ryegrass** | 98.25 (±0.76) de | 98.25 (±0.76) de |
| **2,4-D** | | 99.00 (±0.76) de | 98.25 (±0.76) de |
| **Dicamba** | | 98.75 (±0.76) e | 98.25 (±0.76) de |
| **Aminopyralid** | | 46.00 (±0.52) bc | 46.75 (±0.52) c |
| **2,4-D + Clopyralid** | | 98.50 (±0.76). e | 96.75 (±0.76) de |
| **Dicamba + 2,4-D** | | 99.00 (±0.76) de | 99.00 (±0.76) de |

| Continued | | | |
|---|---|---|---|
| **2,4-D (2X)** | **Italian Ryegrass** | 98.00 (±0.76) de | 99.50 (±0.77) de |
| **Dicamba (2X)** | | 97.25 (±0.76) de | 96.00 (±0.75) de |
| **Halauxifen-methyl** | | 97.00 (±0.76) de | 99.00 (±0.76) de |
| **Control** | **Tall Fescue** | 96.75 (±0.76) de | 96.5 (±0.75) de |
| **2,4-D** | | 93.25 (±0.74) de | 96.75 (±0.76) de |
| **Dicamba** | | 95.00 (±0.75) de | 94.75 (±0.75) de |
| **Aminopyralid** | | 24.00 (±0.38) a | 42.25 (±0.50) b |
| **2,4-D + Clopyralid** | | 94.50 (±0.75) de | 95.75 (±0.75) de |
| **Dicamba + 2,4-D** | | 95.25 (±0.75) de | 92.25 (±0.74) d |
| **2,4-D (2X)** | | 95.50 (±0.75) de | 91.75 (±0.74) de |
| **Dicamba (2X)** | | 95.00 (±0.75) de | 94.50 (±0.75) de |
| **Halauxifen-methyl** | | 96.25 (±0.75) de | 96.75 (±0.76) de |

Table B-42. Seed weight of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Dallas, OR 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | **Growth Stage** | |
|---|---|---|---|
| | | **Anthesis** | **Boot** |
| **Treatment** | **Species** | **Seed weight (SE)** | |
| | | ---------- g/1,000 seeds ---------- | |
| **Control** | **Italian Ryegrass** | 2.212 (±0.051) a | 2.212 (±0.051) a |
| **2,4-D** | | 2.278 (±0.074) a | 2.278 (±0.074) a |
| **Dicamba** | | 2.140 (±0.071) a | 2.140 (±0.071) a |
| **2,4-D + Clopyralid** | | 2.092 (±0.071) a | 2.092 (±0.071) a |
| **Dicamba + 2,4-D** | | 2.200 (±0.072) a | 2.200 (±0.072) a |
| **Control** | **Tall Fescue** | 2.509 (±0.055) a | 2.509 (±0.055) a |
| **2,4-D** | | 2.467 (±0.077) a | 2.467 (±0.077) a |
| **Dicamba** | | 2.452 (±0.076) a | 2.452 (±0.076) a |
| **2,4-D + Clopyralid** | | 2.487 (±0.077) a | 2.487 (±0.077) a |
| **Dicamba + 2,4-D** | | 2.435 (±0.076) a | 2.435 (±0.076) a |

Table B-43. Seed weight of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Corvallis, OR Hyslop farm 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatment | Species | Seed weight (SE) | |
| | | ---------- g/1,000 seeds ---------- | |
| **Control** | **Italian Ryegrass** | 2.130 (±0.059) ce | 2.126 (±0.059) de |
| **2,4-D** | | 2.244 (±0.061) ce | 2.244 (±0.061) de |
| **Dicamba** | | 2.039 (±0.057) ce | 2.100 (±0.058) de |
| **Aminopyralid** | | 1.733 (±0.052) bd | 1.715 (±0.052) abc |
| **2,4-D + Clopyralid** | | 2.200 (±0.060) ce | 2.276 (±0.061) de |
| **Dicamba + 2,4-D** | | 2.172 (±0.060) ce | 2.172 (±0.060) de |
| **Control** | **Tall Fescue** | 2.422 (±0.064) e | 2.422 (±0.064) e |
| **2,4-D** | | 2.332 (±0.062) e | 2.332 (±0.062) e |
| **Dicamba** | | 2.301 (±0.062) ce | 2.301 (±0.062) de |
| **Aminopyralid** | | 1.376 (±0.045) a | 1.413 (±0.046) ab |
| **2,4-D + Clopyralid** | | 2.338 (±0.062) e | 2.347 (±0.062) e |
| **Dicamba + 2,4-D** | | 2.320 (±0.062) e | 2.320 (±0.062) de |

Table B-44. Seed weight of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Gaston, OR 2018 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatments | Species | Seed weight (SE) | |
| | | ---------- g/1,000 seeds ---------- | |
| **Control** | **Italian Ryegrass** | 2.031 (±0.052) b | 2.023 (±0.052) b |
| **2,4-D** | | 1.986 (±0.052) b | 1.979 (±0.051) b |
| **Dicamba** | | 2.050 (±0.052) b | 1.992 (±0.052) b |
| **Aminopyralid** | | 1.334 (±0.042) a | 1.311 (±0.042) a |
| **2,4-D + Clopyralid** | | 2.036 (±0.052) b | 2.016 (±0.052) b |
| **Dicamba + 2,4-D** | | 2.024 (±0.052) b | 2.008 (±0.052) b |
| **2,4-D (2X)** | | 2.007 (±0.052) b | 2.016 (±0.052) b |
| **Dicamba (2X)** | | 2.000 (±0.052) b | 2.002 (±0.052) b |
| **Halauxifen-methyl** | | 2.068 (±0.053) b | 1.986 (±0.052) b |
| **Control** | **Tall fescue** | 2.075 (±0.053) b | 2.031 (±0.052) b |
| **2,4-D** | | 2.060 (±0.052) b | 2.056 (±0.052) b |

| Continued | | | |
|---|---|---|---|
| **Dicamba** | **Tall fescue** | 2.059 (±0.052) b | 2.049 (±0.052) b |
| **Aminopyralid** | | 1.159 (±0.039) a | 1.308 (±0.042) a |
| **2,4-D + Clopyralid** | | 1.965 (±0.051) b | 2.106 (±0.053) b |
| **Dicamba + 2,4-D** | | 2.084 (±0.053) b | 2.064 (±0.053) b |
| **2,4-D (2X)** | | 2.018 (±0.052) b | 2.091 (±0.053) b |
| **Dicamba (2X)** | | 2.054 (±0.052) b | 2.033 (±0.052) b |
| **Halauxifen-methyl** | | 2.028 (±0.052) b | 2.085 (±0.053) b |

Table B-45. Seed weight of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Corvallis, OR Schmidt farm 2018 trial (letters represent different groups on HSD Tukey test p-value < 0.05)

| | | **Growth Stage** | |
|---|---|---|---|
| | | **Anthesis** | **Boot** |
| **Treatments** | **Species** | **Seed weight (SE)** | |
| | | ---------- g/1,000 seeds ---------- | |
| **Control** | **Italian Ryegrass** | 2.006 (±0.025) c | 2.003 (±0.025) c |
| **2,4-D** | | 1.970 (±0.025) c | 2.043 (±0.025) c |
| **Dicamba** | | 2.006 (±0.025) c | 2.021 (±0.025) c |
| **Aminopyralid** | | 1.210 (±0.020) ab | 1.234 (±0.020) b |
| **2,4-D + Clopyralid** | | 1.982 (±0.025) c | 2.023 (±0.025) c |
| **Dicamba + 2,4-D** | | 1.985 (±0.025) c | 2.008 (±0.025) c |
| **2,4-D (2X)** | | 1.981 (±0.025) c | 1.996 (±0.025) c |
| **Dicamba (2X)** | | 2.006 (±0.025) c | 1.980 (±0.025) c |
| **Halauxifen-methyl** | | 2.014 (±0.025) c | 2.040 (±0.025) c |
| **Control** | **Tall Fescue** | 2.018 (±0.025) c | 2.023 (±0.025) c |
| **2,4-D** | | 2.018 (±0.025) c | 2.009 (±0.025) c |
| **Dicamba** | | 2.019 (±0.025) c | 2.043 (±0.025) c |
| **Aminopyralid** | | 1.135 (±0.019) ab | 1.052 (±0.018) a |
| **2,4-D + Clopyralid** | | 2.026 (±0.025) c | 2.010 (±0.025) c |
| **Dicamba + 2,4-D** | | 2.010 (±0.025) c | 2.028 (±0.025) c |
| **2,4-D (2X)** | | 2.018 (±0.025) c | 2.025 (±0.025) c |
| **Dicamba (2X)** | | 1.991 (±0.025) c | 2.031 (±0.025) c |
| **Halauxifen-methyl** | | 2.016 (±0.025) c | 2.031 (±0.025) c |

Table B-46. Seed viability reduction after aging test of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Dallas, OR 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatment | Species | Viability reduction (SE) | |
| | | % | |
| **Control** | **Italian Ryegrass** | 1.90 (±0.74) a | 2.48 (±0.90) a |
| **2,4-D** | | 4.93 (±2.23) a | 0.00 (±0.00) a |
| **Dicamba** | | 2.24 (±1.40) a | 3.54 (±1.85) a |
| **2,4-D + Clopyralid** | | 5.60 (±2.42) a | 2.36 (±1.47) a |
| **Dicamba + 2,4-D** | | 0.90 (±0.85) a | 0.00 (±0.00) a |
| **Control** | **Tall Fescue** | 5.34 (±1.58) a | 8.38 (±2.27) a |
| **2,4-D** | | 6.95 (±2.85) a | 6.07 (±2.60) a |
| **Dicamba** | | 5.29 (±2.41) a | 4.13 (±2.05) a |
| **2,4-D + Clopyralid** | | 5.96 (±2.59) a | 0.73 (±0.79) a |
| **Dicamba + 2,4-D** | | 3.23 (±1.79) a | 4.62 (±2.19) a |

Table B-47. Seed viability reduction after aging test of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Corvallis, OR Hyslop farm 2017 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatment | Species | Viability reduction (SE) | |
| | | % | |
| **Control** | **Italian Ryegrass** | 0.25 (±0.32) a | 0.00 (±0.00) a |
| **2,4-D** | | 0.00 (±0.00) a | 0.00 (±0.00) a |
| **Dicamba** | | 1.00 (±0.66) a | 0.48 (±0.43) a |
| **Aminopyralid** | | 4.74 (±1.73) b | 10.71 (±3.15) c |
| **2,4-D + Clopyralid** | | 0.50 (±0.46) a | 0.00 (±0.00) a |
| **Dicamba + 2,4-D** | | 0.50 (±0.46) a | 0.71 (±0.54) a |
| **Control** | **Tall Fescue** | 0.24 (±0.30) a | 0.00 (±0.00) a |
| **2,4-D** | | 0.71 (±0.54) a | 0.97 (±0.65) a |
| **Dicamba** | | 0.71 (±0.54) a | 0.49 (±0.44) a |
| **Aminopyralid** | | 1.18 (±0.72) a | 0.24 (±0.31) a |
| **2,4-D + Clopyralid** | | 0.00 (±0.00) a | 1.22 (±0.74) a |
| **Dicamba + 2,4-D** | | 1.18 (±0.72) a | 0.73 (±0.55) a |

Table B-48. Seed viability reduction after aging test of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Gaston, OR 2018 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatments | Species | Viability reduction (SE) | |
| | | ---------- % ---------- | |
| **Control** | **Italian Ryegrass** | 2.99 (±1.00) a | 0.99 (±0.57) a |
| **2,4-D** | | 3.49 (±1.09) a | 5.46 (±1.38) a |
| **Dicamba** | | 0.25 (±0.28) a | 2.23 (±0.86) a |
| **Aminopyralid** | | 1.00 (±0.57) a | 10.18 (±1.95) a |
| **2,4-D + Clopyralid** | | 2.50 (±0.91) a | 2.23 (±0.86) a |
| **Dicamba + 2,4-D** | | 3.74 (±1.13) a | 4.96 (±1.31) a |
| **2,4-D (2X)** | | 0.75 (±0.49) a | 7.20 (±1.60) a |
| **Dicamba (2X)** | | 2.25 (±0.86) a | 0.00 (±0.00) a |
| **Halauxifen-methyl** | | 2.50 (±0.91) a | 6.45 (±1.51) a |
| **Control** | **Tall Fescue** | 2.25 (±0.86) a | 2.25 (±0.86) a |
| **2,4-D** | | 2.50 (±0.91) a | 3.49 (±1.09) a |
| **Dicamba** | | 2.74 (±0.96) a | 3.24 (±1.05) a |
| **Aminopyralid** | | 0.00 (±0.00) a | 0.50 (±0.40) a |
| **2,4-D + Clopyralid** | | 2.74 (±0.96) a | 2.50 (±0.91) a |
| **Dicamba + 2,4-D** | | 1.25 (±0.64) a | 3.74 (±1.13) a |
| **2,4-D (2X)** | | 2.25 (±0.86) a | 2.50 (±0.91) a |
| **Dicamba (2X)** | | 2.99 (±1.00) a | 1.25 (±0.64) a |
| **Halauxifen-methyl** | | 2.50 (±0.91) a | 2.50 (±0.91) a |

Table B-49. Seed viability reduction after aging test of the two tested grass species after synthetic auxin herbicide treatment applied at two growth stages in Corvallis, OR Schmidt farm 2018 trial (letters represent different groups on HSD Tukey test p-value < 0.05).

| | | Growth Stage | |
|---|---|---|---|
| | | Anthesis | Boot |
| Treatments | Species | Viability reduction (SE) | |
| | | ---------- % ---------- | |
| **Control** | **Italian Ryegrass** | 2.50 (±0.67) ac | 2.75 (±0.70) abcd |
| **2,4-D** | | 3.00 (±0.73) ac | 3.00 (±0.73) abcd |
| **Dicamba** | | 2.50 (±0.67) ac | 3.00 (±0.73) abcd |
| **Aminopyralid** | | 17.25 (±1.75) bd | 4.75 (±0.92) abcd |
| **2,4-D + Clopyralid** | | 3.00 (±0.73) ac | 3.50 (±0.79) abcd |
| **Dicamba + 2,4-D** | | 3.00 (±0.73) ac | 3.00 (±0.73) abcd |
| **2,4-D (2X)** | | 3.00 (±0.73) ac | 3.00 (±0.73) abcd |
| **Dicamba (2X)** | | 3.00 (±0.73). ac | 3.75 (±0.82) abcd |
| **Halauxifen-methyl** | | 3.00 (±0.73) ac | 2.75 (±0.70) abcd |
| **Control** | **Tall Fescue** | 2.75 (±0.70). ac | 2.50 (±0.67) abcd |
| **2,4-D** | | 3.00 (±0.73). ac | 4.25 (±0.87) abcd |
| **Dicamba** | | 1.25 (±0.47). ac | 1.50 (±0.52) ab |
| **Aminopyralid** | | 1.25 (±0.47) ac | 0.75 (±0.36) ab |
| **2,4-D + Clopyralid** | | 0.00 (±0.00) abcd | 1.25 (±0.47) ab |
| **Dicamba + 2,4-D** | | 0.75 (±0.36). ac | 0.00 (±0.00) abcd |
| **2,4-D (2X)** | | 0.75 (±0.36) ac | 0.00 (±0.00) abcd |
| **Dicamba (2X)** | | 1.50 (±0.52) ac | 0.50 (±0.30) ab |
| **Halauxifen-methyl** | | 2.25 (±0.63) ac | 7.25 (±1.13) cd |

Table B-50. Parameters and standard errors (SE) of fitted log-logistic model for speed of germination in Dallas, OR, trial 2017.

| | Growth Stage | Anthesis | | | | |
|---|---|---|---|---|---|---|
| | | Parameter | | | | |
| Species | Treatment | Slope (SE) | MaxGerm (SE) | MaxGerm Ratio | T50 (SE) | T50 Ratio |
| Italian Ryegrass | Control | -2.852 (±0.162) | 0.775 (±0.027) | 1.000 | 7.174 (±0.275) | 1.000 |
| | 2,4-D | -2.307 (±0.215) | 0.965 (±0.083) | 1.245 | 8.873 (±0.868) | 1.237 |
| | Dicamba | -3.879 (±0.261) | 0.703 (±0.026) | 0.907 | 6.174 (±0.193) | 0.861 |
| | 2,4-D + Clopyralid | -1.335 (±0.113) | 1.000 (±0.077) | 1.290 | 8.356 (±1.087) | 1.165 |
| | Dicamba + 2,4-D | -2.898 (±0.225) | 0.768 (±0.034) | 0.991 | 6.680 (±0.327) | 0.931 |
| Tall Fescue | Control | -4.166 (±0.155) | 1.000 (±0.008) | 1.000 | 6.043 (±0.099) | 1.000 |
| | 2,4-D | -4.111 (±0.193) | 1.000 (±0.017) | 1.000 | 6.391 (±0.146) | 1.058 |
| | Dicamba | -4.032 (±0.221) | 1.000 (±0.012) | 1.000 | 5.953 (±0.145) | 0.985 |
| | 2,4-D + Clopyralid | -4.775 (±0.255) | 0.960 (±0.011) | 0.960 | 5.421 (±0.108) | 0.897 |
| | Dicamba + 2,4-D | -4.583 (±0.245) | 0.975 (±0.011) | 0.975 | 5.666 (±0.118) | 0.938 |
| | Growth Stage | Boot | | | | |
| | | Parameter | | | | |
| Species | Treatment | Slope (SE) | MaxGerm (SE) | MaxGerm Ratio | T50 (SE) | T50 Ratio |
| Italian Ryegrass | Control | -2.406 (±0.156) | 0.824 (±0.040) | 1.001 | 7.797 (±0.443) | 1.000 |
| | 2,4-D | -2.307 (±0.215) | 0.965 (±0.083) | 1.171 | 8.873 (±0.868) | 1.138 |
| | Dicamba | -3.958 (±0.260) | 0.726 (±0.025) | 0.881 | 6.094 (±0.181) | 0.782 |
| | 2,4-D + Clopyralid | -1.384 (±0.109) | 1.000 (±0.068) | 1.214 | 8.032 (±0.902) | 1.030 |
| | Dicamba + 2,4-D | -2.864 (±0.221) | 0.772 (±0.032) | 0.937 | 6.421 (±0.307) | 0.823 |
| Tall Fescue | Control | -4.166 (±0.155) | 1.000 (±0.008) | 1.000 | 6.043 (±0.099) | 1.000 |
| | 2,4-D | -4.111 (±0.193) | 1.000 (±0.017) | 1.000 | 6.391 (±0.146) | 1.058 |
| | Dicamba | -4.032 (±0.221) | 1.000 (±0.012) | 1.000 | 5.953 (±0.145) | 0.985 |
| | 2,4-D + Clopyralid | -4.775 (±0.255) | 0.960 (±0.011) | 0.960 | 5.421 (±0.108) | 0.897 |
| | Dicamba + 2,4-D | -4.583 (±0.245) | 0.975 (±0.011) | 0.975 | 5.666 (±0.118) | 0.938 |

Table B-51. Parameters and standard errors (SE) of fitted log-logistic model for speed of germination in Corvallis, OR, Hyslop trial 2017

| | Growth Stage | Anthesis | | | | |
|---|---|---|---|---|---|---|
| | | Parameter | | | | |
| **Species** | **Treatment** | **Slope (SE)** | **MaxGerm (SE)** | **MaxGerm Ratio** | **T50 (SE)** | **T50 Ratio** |
| **Italian Ryegrass** | **Control** | -4.802 (±0.169) | 0.807 (±0.014) | 1.000 | 4.513 (±0.004) | 1.000 |
| | **2,4-D** | -4.029 (±0.137) | 0.814 (±0.015) | 1.009 | 4.639 (±0.023) | 1.028 |
| | **Dicamba** | -3.951 (±0.136) | 0.801 (±0.015) | 0.993 | 4.501 (±0.023) | 0.997 |
| | **Aminopyralid** | -2.603 (±0.252) | 0.248 (±0.042) | 0.307 | 8.041 (±0.826) | 1.782 |
| | **2,4-D + Clopyralid** | -5.335 (±0.198) | 0.801 (±0.015) | 0.993 | 4.700 (±0.088) | 1.041 |
| | **Dicamba + 2,4-D** | -5.374 (±0.197) | 0.815 (±0.013) | 1.010 | 4.609 (±0.086) | 1.021 |
| **Tall Fescue** | **Control** | -3.473 (±0.124) | 0.925 (±0.024) | 1.000 | 7.015 (±0.150) | 1.000 |
| | **2,4-D** | -3.558 (±0.127) | 0.888 (±0.022) | 0.960 | 6.808 (±0.132) | 0.970 |
| | **Dicamba** | -3.190 (±0.118) | 0.893 (±0.028) | 0.965 | 6.863 (±0.177) | 0.978 |
| | **Aminopyralid** | -3.606 (±0.704) | 0.062 (±0.012) | 0.067 | 5.125 (±0.496) | 0.731 |
| | **2,4-D + Clopyralid** | -3.461 (±0.130) | 0.870 (±0.026) | 0.941 | 7.052 (±0.160) | 1.005 |
| | **Dicamba + 2,4-D** | -3.047 (±0.114) | 0.928 (±0.033) | 1.003 | 7.138 (±0.217) | 1.018 |
| | **Growth Stage** | **Boot** | | | | |
| | | **Parameter** | | | | |
| **Species** | **Treatment** | **Slope (SE)** | **MaxGerm (SE)** | **MaxGerm Ratio** | **T50 (SE)** | **T50 Ratio** |
| **Italian Ryegrass** | **Control** | -4.802 (±0.259) | 0.917 (±0.014) | 1.000 | 4.623 (±0.094) | 1.000 |
| | **2,4-D** | -4.437 (±0.243) | 0.926 (±0.014) | 1.010 | 4.862 (±0.105) | 1.052 |
| | **Dicamba** | -4.857 (±0.264) | 0.911 (±0.015) | 0.993 | 4.905 (±0.098) | 1.061 |
| | **Aminopyralid** | -2.411 (±0.274) | 0.497 (±0.044) | 0.542 | 7.364 (±0.704) | 1.593 |
| | **2,4-D + Clopyralid** | -5.445 (±0.300) | 0.870 (±0.017) | 0.949 | 4.755 (±0.088) | 1.029 |
| | **Dicamba + 2,4-D** | -5.374 (±0.287) | 0.925 (±0.013) | 1.009 | 4.719 (±0.086) | 1.021 |

| Continued | | | | | | |
|---|---|---|---|---|---|---|
| Tall Fescue | **Control** | -3.493 (±0.215) | 1.021 (±0.024) | 1.000 | 7.066 (±0.236) | 1.000 |
| | **2,4-D** | -3.528 (±0.218) | 0.988 (±0.024) | 0.968 | 6.970 (±0.230) | 0.986 |
| | **Dicamba** | -3.369 (±0.215) | 0.980 (±0.026) | 0.960 | 6.970 (±0.247) | 0.986 |
| | **Aminopyralid** | -4.006 (±0.574) | 0.141 (±0.018) | 0.138 | 5.542 (±0.360) | 0.784 |
| | **2,4-D + Clopyralid** | -3.466 (±0.218) | 0.989 (±0.026) | 0.969 | 7.111 (±0.245) | 1.006 |
| | **Dicamba + 2,4-D** | -2.959 (±0.199) | 1.063 (±0.033) | 1.041 | 7.200 (±0.314) | 1.019 |

Table B-52. Parameters and standard errors (SE) of fitted log-logistic model for speed of germination in Gaston, OR, trial 2018

| **Growth Stage** | | **Anthesis** | | | | |
|---|---|---|---|---|---|---|
| | | **Parameter** | | | | |
| **Species** | **Treatment** | **Slope (SE)** | **MaxGerm (SE)** | **MaxGerm Ratio** | **T50 (SE)** | **T50 Ratio** |
| Italian Ryegrass | **Control** | -1.958 (±0.186) | 0.975 (±0.027) | 1.000 | 3.860 (±0.215) | 1.000 |
| | **2,4-D** | -2.820 (±0.193) | 0.928 (±0.019) | 0.952 | 4.684 (±0.169) | 1.213 |
| | **Dicamba** | -2.703 (±0.209) | 0.876 (±0.038) | 0.898 | 6.913 (±0.372) | 1.791 |
| | **Aminopyralid** | -1.784 (±0.282) | 0.438 (±0.031) | 0.449 | 3.596 (±0.338) | 0.932 |
| | **2,4-D + Clopyralid** | -2.881 (±0.201) | 0.909 (±0.027) | 0.932 | 6.025 (±0.248) | 1.561 |
| | **Dicamba + 2,4-D** | -1.915 (±0.126) | 1.000 (±0.031) | 1.026 | 5.069 (±0.277) | 1.313 |
| | **2,4-D (2X)** | -2.621 (±0.192) | 0.910 (±0.022) | 0.933 | 4.623 (±0.186) | 1.198 |
| | **Dicamba (2X)** | -2.608 (±0.197) | 0.871 (±0.023) | 0.893 | 4.523 (±0.185) | 1.172 |
| | **Halauxifen-methyl** | -2.499 (±0.203) | 0.904 (±0.019) | 0.927 | 3.577 (±0.145) | 0.927 |
| Tall Fescue | **Control** | -4.334 (±0.233) | 0.989 (±0.009) | 1.000 | 5.510 (±0.120) | 1.000 |
| | **2,4-D** | -5.824 (±0.306) | 0.98 (±0.007) | 0.991 | 5.084 (±0.082) | 0.923 |
| | **Dicamba** | -3.848 (±0.218) | 0.989 (±0.013) | 1.000 | 5.863 (±0.151) | 1.064 |
| | **Aminopyralid** | -3.941 (±0.430) | 0.252 (±0.022) | 0.255 | 5.542 (±0.271) | 1.006 |
| | **2,4-D + Clopyralid** | -5.945 (±0.313) | 0.977 (±0.008) | 0.988 | 5.048 (±0.080) | 0.916 |
| | **Dicamba + 2,4-D** | -4.096 (±0.230) | 0.932 (±0.015) | 0.942 | 5.124 (±0.121) | 0.930 |
| | **2,4-D (2X)** | -3.439 (±0.172) | 1.000 (±0.022) | 1.011 | 6.837 (±0.194) | 1.241 |

| | | | Continued | | | |
|---|---|---|---|---|---|---|
| Tall Fescue | Dicamba (2X) | -4.670 (±0.249) | 0.968 (±0.011) | 0.979 | 5.291 (±0.107) | 0.960 |
| | Halauxifen-methyl | -4.100 (±0.223) | 0.990 (±0.009) | 1.001 | 5.027 (±0.114) | 0.912 |
| | Growth Stage | | Boot | | | |
| | | | Parameter | | | |
| Species | Treatment | Slope (SE) | MaxGerm (SE) | MaxGerm Ratio | T50 (SE) | T50 Ratio |
| Italian Ryegrass | Control | -1.937 (±0.186) | 0.993 (±0.025) | 1.000 | 3.724 (±0.205) | 1.000 |
| | 2,4-D | -1.637 (±0.202) | 0.975 (±0.029) | 0.982 | 2.943 (±0.196) | 0.790 |
| | Dicamba | -1.801 (±0.211) | 0.810 (±0.031) | 0.816 | 3.432 (±0.227) | 0.922 |
| | Aminopyralid | -2.205 (±0.228) | 0.624 (±0.038) | 0.628 | 5.744 (±0.437) | 1.542 |
| | 2,4-D + Clopyralid | -2.240 (±0.186) | 0.946 (±0.024) | 0.953 | 4.320 (±0.209) | 1.160 |
| | Dicamba + 2,4-D | -1.660 (±0.188) | 0.956 (±0.042) | 0.963 | 4.185 (±0.339) | 1.124 |
| | 2,4-D (2X) | -2.292 (±0.188) | 0.947 (±0.022) | 0.954 | 4.062 (±0.184) | 1.091 |
| | Dicamba (2X) | -2.125 (±0.186) | 0.946 (±0.027) | 0.953 | 4.280 (±0.224) | 1.149 |
| | Halauxifen-methyl | -2.521 (±0.186) | 0.964 (±0.020) | 0.971 | 4.481 (±0.183) | 1.203 |
| Tall Fescue | Control | -4.334 (±0.233) | 0.990 (±0.009) | 1.000 | 5.510 (±0.120) | 1.000 |
| | 2,4-D | -4.154 (±0.228) | 0.961 (±0.012) | 0.971 | 5.000 (±0.114) | 0.907 |
| | Dicamba | -4.436 (±0.240) | 0.963 (±0.012) | 0.973 | 5.328 (±0.114) | 0.967 |
| | Aminopyralid | -3.905 (±0.306) | 0.504 (±0.026) | 0.509 | 5.879 (±0.210) | 1.067 |
| | 2,4-D + Clopyralid | -4.545 (±0.244) | 0.967 (±0.011) | 0.977 | 5.373 (±0.112) | 0.975 |
| | Dicamba + 2,4-D | -5.002 (±0.265) | 0.961 (±0.011) | 0.971 | 5.116 (±0.096) | 0.928 |
| | 2,4-D (2X) | -5.057 (±0.268) | 0.958 (±0.011) | 0.968 | 5.394 (±0.101) | 0.979 |
| | Dicamba (2X) | -6.750 (±0.373) | 0.943 (±0.012) | 0.953 | 5.023 (±0.072) | 0.912 |
| | Halauxifen-methyl | -4.664 (±0.248) | 0.975 (±0.010) | 0.985 | 5.546 (±0.113) | 1.007 |

Table B-53. Parameters and standard errors (SE) of fitted log-logistic model for speed of germination in Corvallis, OR, Schmidt farm trial 2018

| | **Growth Stage** | **Anthesis** | | | | |
|---|---|---|---|---|---|---|
| | | **Parameter** | | | | |
| **Species** | **Treatment** | **Slope (SE)** | **MaxGerm (SE)** | **MaxGerm Ratio** | **T50 (SE)** | **T50 Ratio** |
| **Italian Ryegrass** | **Control** | -3.120 (±0.179) | 1.000 (±0.010) | 1.000 | 3.910 (±0.116) | 1.000 |
| | **2,4-D** | -3.188 (±0.181) | 1.000 (±0.010) | 1.000 | 3.987 (±0.116) | 1.020 |
| | **Dicamba** | -3.243 (±0.207) | 1.000 (±0.007) | 1.000 | 3.270 (±0.098) | 0.836 |
| | **Aminopyralid** | -1.341 (±0.247) | 0.572 (±0.062) | 0.572 | 4.784 (±0.923) | 1.224 |
| | **2,4-D + Clopyralid** | -2.705 (±0.163) | 1.000 (±0.013) | 1.000 | 3.822 (±0.132) | 0.977 |
| | **Dicamba + 2,4-D** | -3.209 (±0.180) | 1.000 (±0.011) | 1.000 | 3.755 (±0.109) | 0.960 |
| | **2,4-D (2X)** | -3.054 (±0.165) | 1.000 (±0.014) | 1.000 | 4.361 (±0.131) | 1.115 |
| | **Dicamba (2X)** | -2.963 (±0.204) | 0.996 (±0.009) | 0.996 | 3.519 (±0.113) | 0.900 |
| | **Halauxifen-methyl** | -2.606 (±0.173) | 1.000 (±0.013) | 1.000 | 3.710 (±0.134) | 0.949 |
| **Tall Fescue** | **Control** | -3.993 (±0.222) | 0.965 (±0.012) | 1.000 | 4.993 (±0.118) | 1.000 |
| | **2,4-D** | -4.774 (±0.255) | 0.949 (±0.012) | 0.983 | 5.012 (±0.100) | 1.004 |
| | **Dicamba** | -3.536 (±0.216) | 0.907 (±0.016) | 0.940 | 4.106 (±0.113) | 0.822 |
| | **Aminopyralid** | -3.294 (±0.391) | 0.250 (±0.022) | 0.259 | 4.332 (±0.246) | 0.868 |
| | **2,4-D + Clopyralid** | -3.715 (±0.215) | 0.942 (±0.013) | 0.976 | 4.315 (±0.111) | 0.864 |
| | **Dicamba + 2,4-D** | -3.933 (±0.227) | 0.904 (±0.017) | 0.937 | 5.082 (±0.127) | 1.018 |
| | **2,4-D (2X)** | -4.419 (±0.236) | 0.967 (±0.010) | 1.002 | 4.581 (±0.098) | 0.917 |
| | **Dicamba (2X)** | -3.630 (±0.216) | 0.947 (±0.013) | 0.981 | 3.943 (±0.104) | 0.790 |
| | **Halauxifen-methyl** | -5.896 (±0.312) | 0.959 (±0.010) | 0.994 | 4.747 (±0.078) | 0.951 |
| | **Growth Stage** | **Boot** | | | | |
| | | **Parameter** | | | | |
| **Species** | **Treatment** | **Slope (SE)** | **MaxGerm (SE)** | **MaxGerm Ratio** | **T50 (SE)** | **T50 Ratio** |
| **Italian Ryegrass** | **Control** | -3.092 (±0.177) | 1.000 (±0.011) | 1.000 | 3.916 (±0.117) | 1.000 |

| | | | Continued | | | |
|---|---|---|---|---|---|---|
| Italian Ryegrass | **2,4-D** | -3.061 (±0.207) | 0.999 (±0.007) | 0.999 | 3.494 (±0.108) | 0.892 |
| | **Dicamba** | -3.409 (±0.212) | 0.995 (±0.007) | 0.995 | 3.668 (±0.101) | 0.937 |
| | **Aminopyralid** | -1.858 (±0.255) | 0.515 (±0.033) | 0.515 | 3.952 (±0.337) | 1.009 |
| | **2,4-D + Clopyralid** | -2.842 (±0.187) | 1.000 (±0.011) | 1.000 | 3.966 (±0.131) | 1.013 |
| | **Dicamba + 2,4-D** | -3.151 (±0.182) | 1.000 (±0.010) | 1.000 | 3.752 (±0.111) | 0.958 |
| | **2,4-D (2X)** | -3.346 (±0.187) | 1.000 (±0.050) | 1.000 | 3.515 (±0.099) | 0.898 |
| | **Dicamba (2X)** | -2.772 (±0.191) | 0.977 (±0.015) | 0.977 | 4.143 (±0.144) | 1.058 |
| | **Halauxifen-methyl** | -3.324 (±0.204) | 0.996 (±0.008) | 0.996 | 3.872 (±0.109) | 0.989 |
| Tall Fescue | **Control** | -3.993 (±0.222) | 0.965 (±0.012) | 1.000 | 4.993 (±0.118) | 1.000 |
| | **2,4-D** | -3.426 (±0.201) | 0.983 (±0.012) | 1.019 | 4.724 (±0.130) | 0.946 |
| | **Dicamba** | -4.732 (±0.256) | 0.923 (±0.014) | 0.956 | 4.765 (±0.097) | 0.954 |
| | **Aminopyralid** | -3.619 (±0.312) | 0.432 (±0.026) | 0.448 | 5.190 (±0.208) | 1.039 |
| | **2,4-D + Clopyralid** | -4.653 (±0.253) | 0.907 (±0.015) | 0.940 | 4.540 (±0.096) | 0.909 |
| | **Dicamba + 2,4-D** | -5.207 (±0.276) | 0.933 (±0.013) | 0.967 | 4.583 (±0.086) | 0.918 |
| | **2,4-D (2X)** | -3.791 (±0.217) | 0.939 (±0.014) | 0.973 | 4.805 (±0.122) | 0.962 |
| | **Dicamba (2X)** | -5.219 (±0.287) | 0.882 (±0.017) | 0.914 | 5.115 (±0.097) | 1.024 |
| | **Halauxifen-methyl** | -4.819 (±0.262) | 0.916 (±0.015) | 0.949 | 5.008 (±0.100) | 1.003 |