# DISTRIBUTION AND FREQUENCY OF ITALIAN RYEGRASS HERBICIDE RESISTANCE IN THE WILLAMETTE VALLEY

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**Introduction**

Weed management is one of the most challenging parts of any agricultural system, and herbicide resistance plays a significant role in management decision-making. Herbicide-resistant Italian ryegrass (*Lolium perenne* spp. multiflorum) is a management issue for growers worldwide, with more than 200 cases of unique resistant biotypes reported (Heap, 2018).

The vast majority of Oregon's Willamette Valley agriculture is based on grass seed production, which requires growers to achieve a premium level of seed purity. Without successful weed control, seed lots can be contaminated with Italian ryegrass seeds that carry herbicide resistance traits. Knowing the current distribution of herbicide-resistant biotypes and how commonly they can be found can help growers avoid contamination and prioritize management of resistant biotypes.

Field surveys have been done in many states and countries to understand the distribution and frequency of a weed and resistant biotypes (Hanson et al., 2009; Hanzlik and Gerowitt, 2016; Lutman et al., 2009; Owen and Powles, 2009). However, this type of study has not been done in Oregon with Italian ryegrass. The objective of this study was to document the distribution and frequency of multiple types of herbicide-resistant Italian ryegrass biotypes in western Oregon.

**Materials and Methods**

A 2-year survey was conducted during the summers of 2017 and 2018 in the ecoregion of the Willamette Valley in Oregon. The sites surveyed were randomly selected using available data from USDA CropScape regarding crop geospatial location (Han et al., 2012). For each year of the survey, a different randomization was utilized. The focus was to select fields where tall fescue or wheat crops were present. However, CropScape data were not precise enough to differentiate grass species, and many of the randomized geographic locations were grass species other than tall fescue and even other types of crops. These locations in other crops were surveyed, but if the field contained annual or perennial ryegrass as a crop, it was not included in the survey. Each site was at least 2 miles from the nearest site to best represent the surveyed area and also to determine whether there were any spatial clusters of herbicide resistance.

This survey used a stratified randomized design, with the Willamette Valley divided into three strata (north, central, and south) according to agricultural land acreage of the crops of interest. Four hundred fifty sites were randomly selected, and 150 of these sites were surveyed. At each site, the following information was collected: presence of the weed, Italian ryegrass density level (high = 20 or more plants/m$^2$; medium = 10–19 plants/m$^2$; low = fewer than 10 plants/m$^2$), GPS coordinates, and type of crop cultivated.

If Italian ryegrass was present, seed heads were collected, and progeny of these seeds were tested for herbicide resistance under greenhouse and laboratory conditions. Nine herbicides were tested for resistance, including six postemergent (Table 1) and three preemergent (Table 2) products.

Table 1. Postemergent herbicides and rates used for greenhouse screening test.

| WSSA group | Active ingredient | Mode of action[1] | Trade name | Rate |
|---|---|---|---|---|
| | | | | (lb/a) |
| 1 | Quizalofop-P-ethyl | ACCase inhibitor | Assure II | 0.750 |
| 1 | Clethodim | ACCase inhibitor | SelectMax | 1.000 |
| 1 | Pinoxaden | ACCase inhibitor | Axial XL | 1.020 |
| 2 | Pyroxsulam | ALS inhibitor | Powerflex HL | 0.125 |
| 9 | Glyphosate | EPSPs inhibitor | Makaze | 2.000 |
| 22 | Paraquat | PS I inhibitor | Gramoxone SL 2.0 | 0.250 |

[1]ACCase = acetyl-CoA carboxylase enzyme; ALS = acetolactate synthase; EPSPs = 5-enolpyruvylshikimate-3-phosphate synthase; PS I = photosystem I

Table 2. Preemergent herbicides and rates used for resistance screening in laboratory.

| WSSA group | Active ingredient | Mode of action[1] | Trade name | Rate |
|---|---|---|---|---|
| | | | | (µM) |
| 15 + 5 | Flufenacet + metribuzin | VLCFA inhibitor | Axiom | 2.20 |
| 15 | Pyroxasulfone | VLCFA inhibitor | Zidua | 0.98 |
| 5 | Pronamide | Microtubule assembly inhibition | Kerb | 1.40 |

[1]VLCFA = very-long-chain fatty acid

The postemergence resistance screening test was done in the greenhouse using a complete randomized design with four replications. Each replication consisted of a square tray (9 inches x 9 inches ), with 16 Italian ryegrass plants per tray. Plants were sprayed when the seedlings reached an average height of 6 inches and the two-leaf growth stage. Visual survival rate and image analysis for green area measurement were collected 21 days after treatment.

Preemergence resistance screening was completed using a seed germination assay in germination boxes (4⁵⁄₁₆ inches x 4⁵⁄₁₆ inches x 1⅜ inches) containing 1% agarose media with a known rate of each herbicide (Murray et al., 1996). Application rates were determined by a dose-response assay with known susceptible populations. Four replications with 16 seeds per germination box were used in complete randomized design. Visual germination rate and image analysis for green area measurement were collected 12 days after seed placement in the media.

Resistance levels were classified as susceptible (0–2% survival), developing resistance (2–19% survival), and resistant (20% or more survival). This classification was made based on survival rate and green leaf-area reduction compared to an untreated control. For fields where Italian ryegrass was not found, it was assumed that if any Italian ryegrass was present in the field it was controlled during the season and therefore classified as susceptible. Resistance types were classified as multiple (resistance to more than one mode of action), cross (resistance to more than one herbicide that acts at the same site of action), and single (resistance to only one specific herbicide).

A binomial logistic analysis was used to check whether the probability of finding the weed present and herbicide resistance was related to altitude, location, or type of crop. The frequency of weed presence and resistance was calculated using the Survey package with R software. A multispatial cluster analysis and a nearest neighbor spatial analysis were made using ArcGIS to determine whether any spatial clusters of herbicide resistance were present.

**Results and Discussion**

Overall, Italian ryegrass was present in 75 of the 150 fields surveyed during the 2-year study (Tables 3 and 4). Regarding density, 21.33% of the surveyed fields had a high density (20 plants or more/m$^2$), 10.67% had a medium density (10–19 plants/m$^2$), and 18% had a low density (fewer than 10 plants/m$^2$). With respect to herbicide resistance, 36.67% of the fields surveyed had some form of herbicide resistance present (Figure 1).

Table 3. Frequency of Italian ryegrass presence in surveyed counties.

| County | Not present | Present | Total | Frequency |
|---|---|---|---|---|
| | -------------- (no.) | -------------- | | (%) |
| Benton | 27 | 21 | 48 | 43.75 |
| Clackamas | 0 | 1 | 1 | 100.00 |
| Linn | 4 | 8 | 12 | 66.67 |
| Marion | 20 | 18 | 38 | 47.37 |
| Polk | 7 | 8 | 15 | 53.33 |
| Washington | 9 | 9 | 18 | 50.00 |
| Yamhill | 8 | 10 | 18 | 55.56 |
| Total | 75 | 75 | 150 | 50.00 |

Table 4. Frequency of Italian ryegrass presence in each stratum.

| Stratum | Not present | Present | Total | Frequency |
|---|---|---|---|---|
| | --------------- (no.) | -------------- | | (%) |
| Center | 25 | 25 | 50 | 50 |
| North | 22 | 28 | 50 | 56 |
| South | 28 | 22 | 50 | 44 |
| Total | 75 | 75 | 150 | 50 |

Regarding presence of multiple-resistant biotypes, 23.33% carried resistance traits to more than one mode of action. The most common type of herbicide resistance was to herbicides with the mode of action known as ACCase (acetyl-CoA carboxylase enzyme) inhibitors; 25% of the surveyed fields had resistant traits present to at least one herbicide of this group (Figure 2). The most common multiple-resistance combination was to herbicides in the ACCase and ALS (acetolactate synthase) inhibitor mode of action groups (Figure 3). These results confirm what growers and agronomists have observed in the field for many years regarding herbicides in the EPSPs, ALS, and ACCase inhibitor mode of action groups. These products are no longer providing good control of Italian ryegrass, indicating widespread herbicide resistance throughout the Willamette Valley.

Among the herbicides tested in the study, resistance levels varied (Figure 4). The most effective herbicide tested was pyroxasulfone, which controlled 99% of all tested populations. To date, no resistance has been documented to pyroxasulfone, and it is possible this active ingredient will be relied upon in the future to control resistant populations.

The density of Italian ryegrass plants varied by stratum (data not shown). Where Italian ryegrass was present, 42.67% of locations had a high density of Italian ryegrass present, while 36% and 21.33% of locations had low and medium levels of Italian ryegrass density, respectively. Herbicide resistance was present in all three density levels and was unrelated to density level.

The average nearest neighbor ratio analysis showed that significant spatial clusters were found with respect to the presence of multiple resistance, indicating that some regions of the Valley present a concentration of this type of resistance

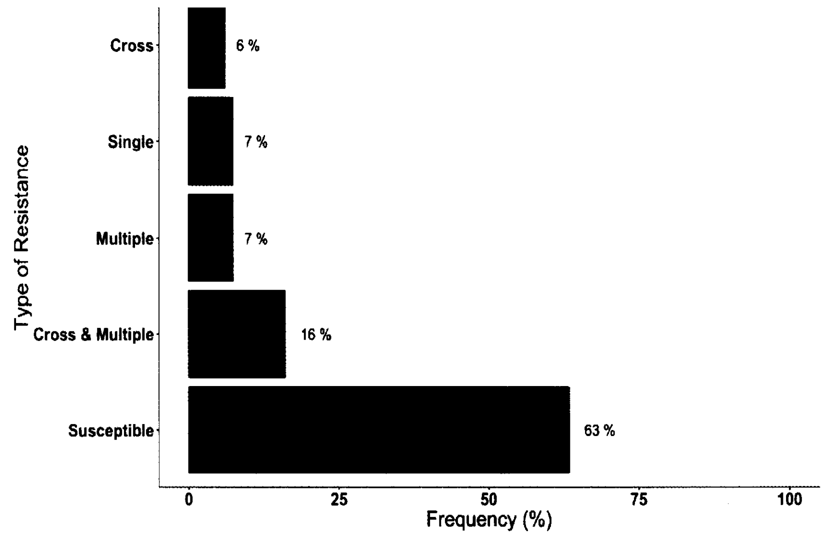


Figure 1. Frequency of presence of different types of herbicide resistance in Italian ryegrass.

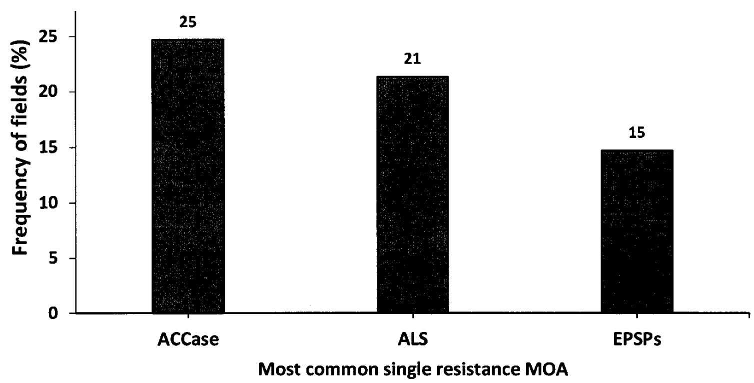


Figure 2. Frequency of the most common mode of action (MOA) resistance cases in Italian ryegrass.

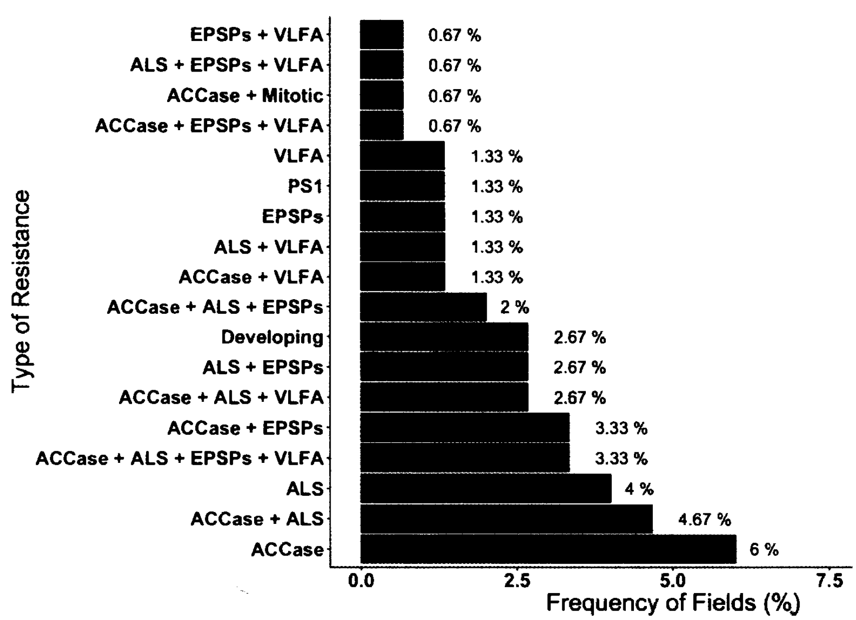


Figure 3. Frequency of specific types of single and multiple resistance in populations of Italian ryegrass in surveyed fields.

(data not shown). In contrast to one of our hypotheses, the binomial-logistic analysis indicated that Italian ryegrass presence and resistance to any herbicide is not related to the specific stratum location, altitude, or type of crop (data not shown). Future studies should focus on trying to understand what factors affect the presence and proportion of certain types of herbicide resistance in a given area.

**Conclusion**

Results from this study indicate widespread distribution of herbicide-resistant Italian ryegrass in the Willamette Valley, with some populations exhibiting resistance to multiple herbicide mode of action groups. It is theorized that these resistant populations developed over many years due to intense selection pressure resulting from repeated application of herbicides with similar modes of action. However, the herbicide assay revealed alternative and effective herbicide options, such as pyroxasulfone, which could help reduce resistant Italian ryegrass populations.

Some specific locations presented clusters of populations with multiple-resistance traits, indicating that some regions are selecting for multiple-resistant populations.

To our knowledge, this is the first study in Oregon that shows the current status of herbicide-resistant Italian ryegrass in the Willamette Valley. It should provide the basis for future studies to investigate factors that influence mechanisms of resistance in the Willamette Valley.

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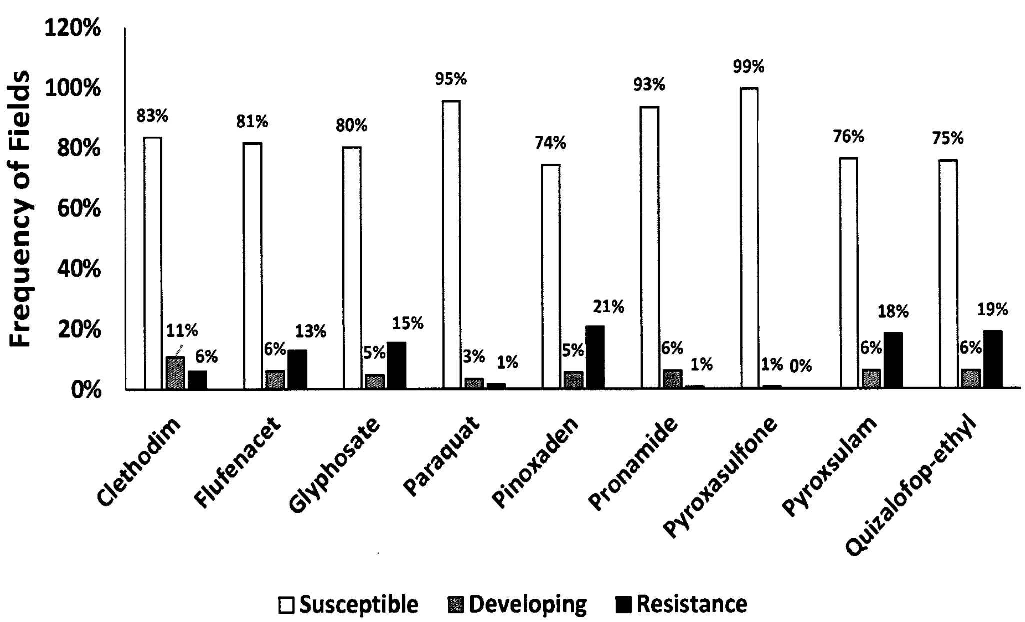

Figure 4. Frequency of fields with different levels of resistance to herbicides.