

## ACCase cross-resistance in Italian ryegrass (*Lolium multiflorum*) sub-populations from Oregon, US

ACCase-Kreuzresistenz bei Subpopulationen des Italienischen Weidelgrases (*Lolium multiflorum*) in Oregon, USA

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### Summary

ACCase-resistant Italian ryegrass (*Lolium multiflorum*) is one of the most troublesome weeds in US wheat production systems. This study had the objective of determining the resistance pattern of three sub-populations selected from a winter wheat field with history of ACCase-inhibitor herbicides applications in Oregon. A greenhouse experiment was conducted to test four ACCase-inhibitor herbicides for the control of the three sub-populations: Pinoxaden, clethodim, sethoxydim and clodinafop. All three sub-populations were cross-resistant to the commercial dose of at least two herbicides. One sub-population was cross-resistant to all herbicides tested. This resistance pattern highlights the need of studying different ACCase herbicides for cross-resistance, as well as herbicides with different modes of action for multiple-resistance to control these sub-populations.

**Keywords:** Acetyl-coA carboxylase, aryloxyphenoxypropionate, cyclohexanodione, herbicide resistance

### Zusammenfassung

ACCase-resistentes Italienisches Weidelgras (*Lolium multiflorum*) ist eines der problematischsten Ungräser in nordamerikanischen Weizenanbausystemen. Ziel dieser Arbeit ist die Bestimmung des Resistenzmusters dreier Subpopulationen, die aus einem Winterweizenfeld mit einer ausgeprägten Historie von Herbizidapplikationen mit ACCase-Inhibitoren stammten. Ein Gewächshausversuch wurde durchgeführt um die Bekämpfung der drei Subpopulationen mit vier ACCase-Inhibitoren zu untersuchen: Pinoxaden, Clethodim, Sethoxydim und Clodinafop. Alle drei Subpopulationen zeigten bei einer Applikation mit der empfohlenen Aufwandmenge Kreuzresistenzen gegen mindestens zwei der getesteten Wirkstoffe. Eine Subpopulation erwies sich als kreuzresistent gegen alle vier getesteten Wirkstoffe. Die beobachteten Resistenzmuster unterstreichen die dringende Notwendigkeit der Erforschung verschiedener ACCase-Inhibitoren bezüglich ihrer Kreuzresistenzmuster, sowie die Untersuchung von Herbiziden mit unterschiedlichen Wirkungsweisen auf vorhandene multiple Resistenzen mit dem Ziel der Bekämpfung dieser Subpopulationen.

**Stichwörter:** Acetyl-coA-carboxylase, Aryloxyphenoxypropionate, Cyclohexanodione, Herbizidresistenz

### 1. Introduction

Italian ryegrass (*Lolium multiflorum* L.) is an annual, outcrossing weed species that infests cereal fields and orchards, particularly in the Pacific Northwest and Southeastern United States. It was introduced into North America from Europe as forage grass (LEIBL and WORSHAM, 1987). Since then, this species has become a weed in winter cereal crops, such as wheat, grass seed crops and hazelnut orchards (STANGER and APPLEBY, 1989; PEREZ-JONES et al., 2005). Chemical control is the only effective practical strategy to control *L. multiflorum* in wheat (BAILEY and WILSON, 2003). Herbicides that inhibit the enzyme Acetyl-CoA carboxylase (ACCase inhibitors) were first introduced in the late 1970's, and provide excellent control of grass weeds in both cereal and broadleaf crops (DEVINE and SHIMABUKURO, 1994). ACCase inhibitors are also known as HRAC group A herbicides or group 1 herbicides (MALLORY-SMITH and RETZINGER, 2003). There are three chemical classes for ACCase-inhibitor herbicides: Aryloxyphenoxypropionates (APPs), cyclohexanediones (CHDs) and phenylpyrazolines (PPZ). The primary target site for APP and CHD herbicides is the CT domain of the plastidic ACCase, which has two regions that are critical for sensitivity to these herbicides (DELYE et al., 2005). Pinoxaden (PPZ), a phenylpyrazoline herbicide introduced in 2006, is a relatively new class of ACCase inhibitors. Pinoxaden is also applied post-emergence and is effective in controlling several grass species, including *L. multiflorum* in wheat and other crops. Good crop tolerance to these herbicides, coupled

with the excellent efficacy on several grass weed species and the introduction of a wide number of graminicides, led to the widespread and repeated use of these herbicides in a variety of crops (DEVINE and SHIMABUKURO, 1994). Resistance to ACCase inhibitors was first reported in 1982 (HEAP and KNIGHT, 1986) and a total of 41 weed species have been identified with resistance to ACCase inhibitors worldwide. Worldwide, there has been an increase in the resistance to the ACCase inhibiting aryloxyphenoxypropionate (APP) and cyclohexanodione (CHD). The appearance of resistance to ACCase inhibitors in a number of grass weed species including *Alopecurus myosuroides* (DELYE et al., 2005), *Lolium multiflorum* (KUK et al., 2008) and *Avena fatua* (ULUDAG et al., 2008) has been attributed to the repeated use of APP or CHD herbicides. Three principal mechanisms have been proposed to explain resistance to the APP or CHD herbicides in weed biotypes (DEVINE and SHIMABUKURO, 1994). An enhanced ability to metabolize the herbicide, which has implicated two types of enzyme, cytochrome P450 monooxygenase and glutathione S-transferases, was determined to be the mechanism of resistance in a biotype of *Digitaria sanguinalis* (HIDAYAT and PRESTON, 1997) and two biotypes of *Alopecurus myosuroides* (HALL et al., 1997; MENEZES and DEPRADO, 1996). Elevated levels of the enzyme glutathione transferase contributed to the resistance of *A. myosuroides* biotypes to the APP herbicide fenoxaprop-ethyl (CUMMINS et al., 1997). Increased herbicide metabolism also played some role in the resistance to ACCase inhibitors in *A. fatua* (MANEECHOTE et al., 1997) and *Lolium rigidum* (HOLTUM et al., 1991) biotypes. Recovery of diclofop-induced depolarization of cell membrane potential after removal of the herbicide from the external medium was found to be correlated with resistance in *L. rigidum* biotypes from Spain and Australia (DEPRADO et al., 1999; HAUSLER et al., 1991). More studies are necessary to determine if such membrane recovery response provides a resistance mechanism or has only a secondary effect. SEEFELDT et al. (1996) found that the resistance in two *A. fatua* biotypes was due to ACCase alteration rather than differential absorption, translocation, or metabolism of diclofop. To date, no resistant weed biotypes have been identified that exhibit differences in absorption or translocation of APP or CHD herbicides when compared to susceptible biotypes (DEVINE, 1997). In most cases where the resistance mechanism has been determined, it is due to the presence of an insensitive form of ACCase caused by point mutations in the gene encoding the CT domain of plastidic ACCase (DEVINE, 1997). ACCase-resistance in *Lolium* spp. threatens cereal production in Australia, Canada, Chile, France, Saudi Arabia, South Africa, Spain, the United Kingdom, and the USA (HEAP, 1999). Therefore, this species evolved resistance to ACCase herbicides within a short period. Greenhouse experiments were conducted in order to identify resistance patterns in a *L. multiflorum* population from a winter wheat field from Oregon to CHD, APP and PPZ classes of herbicides.

## 2. Materials and methods

### 2.1 Plant Material

Two populations of *L. multiflorum* were used in this study: A known susceptible (S) *L. multiflorum* and a suspected resistant (R) *L. multiflorum* sub-population originally from a winter wheat field from Yamhill County, Oregon, USA. On this field, ACCase-inhibitor herbicides had been used to control *L. multiflorum* repeatedly over five years.

### 2.2 Whole-plant bioassay

Seeds from the resistant *L. multiflorum* population were collected in the field. A greenhouse herbicide screening was conducted from February 2009 to April 2009 at Oregon State University, USA, with the objective of confirming resistance. Seeds were germinated in 27.30 cm x 53.98 cm trays containing commercial potting mix (Sunshine Mix #1, Sun Gro Horticulture Inc, Bellevue, WA, USA). Water was provided as necessary and plants were grown under greenhouse conditions of 32/25 °C day/night temperature and photoperiod of 14 hours. Herbicide treatments were applied at the two-leaf growth stage, using an experimental sprayer delivering 185 l/ha at 200 kPa. Plants were treated with doses corresponding to the recommended field rate (g a.i./ha) of four ACCase inhibitor herbicides (Tab. 1). At 30 days after treatment (DAT), plants were visually evaluated, classified and counted as dead or alive. Surviving plants were classified in three groups based on their resistance patterns. Each group

was defined as a sub-population. A sub-population was considered resistant when mortality was less than 50 %. The experiment was arranged in a randomized complete block design with three replications and was conducted twice.

**Tab. 1** Herbicides and the respective doses (g a.i./ha) used in the experiment.

**Tab. 1** *Herbizide und die entsprechenden Aufwandmengen (g a.i./ha), die im Experiment eingesetzt wurden.*

Pinoxaden	59.4
Clethodim	140.1
Sethoxydim	235.4
Clodinafop	70

### 3. Results

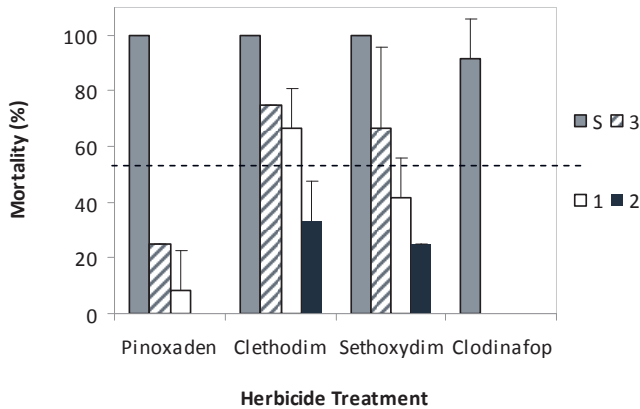
#### 3.1 Single-dose whole-plant experiment for confirmation of herbicide resistance

Visual evaluation at 30 DAT with the ACCase-inhibitor herbicides identified three resistant sub-populations at the recommended field rate of each herbicide. The susceptible *L. multiflorum* population was effectively controlled by all four herbicides. The three sub-populations (1, 2 and 3) were resistant to at least two of the four herbicides tested (Tab. 2; Fig. 1). For the APP herbicide clodinafop and the PPZ herbicide pinoxaden, plant mortality was 0 for all sub-populations. For the PPZ herbicide pinoxaden, plant mortality was 8 %, 0 % and 25 % for sub-populations 1, 2 and 3, respectively. Sub-populations 1 and 3 were susceptible to the CHD herbicide clethodim and had 67 % and 75 % mortality, respectively. Sub-population 1 had a lower resistance level when compared with the other sub-populations, with 42 % mortality when treated with sethoxydim. Sub-population 2 was resistant to all four herbicides tested, and had 0 %, 33 %, 25 % and 0 % mortality for the herbicides pinoxaden, clethodim, sethoxydim and clodinafop, respectively. Sub-population 3 also was susceptible when treated with sethoxydim with 67 % mortality. Clethodim was the most effective herbicide for control of the *L. multiflorum* sub-populations compared to the other herbicides tested. Sub-population 2 was the only one resistant to clethodim.

**Tab. 2** Patterns of ACCase-inhibiting herbicide resistance in *L. multiflorum*.

**Tab. 2** *Muster der Resistenz bei ACCase-Inhibitoren und *L. multiflorum*.*

	Pinoxaden	Clethodim	Sethoxydim	Clodinafop
1	R	S	R	R
2	R	R	R	R
3	R	S	S	R
S	S	S	S	S



**Fig. 1** *L. multiflorum* mortality percentage per ACCase inhibitor herbicide by sub-population at 30 DAT. Dashed line indicates 50 % mortality.

**Abb. 1** Prozent Mortalität bei drei Sub-Populationen von *L. multiflorum* nach Applikation mit vier ACCase-Inhibitoren, 30 DAT. Die gestrichelte Linie markiert eine Mortalität von 50 %.

#### 4. Discussion

This study confirmed different resistance patterns in a suspected resistant *L. multiflorum* population. These sub-populations are originally from the same winter wheat field from Oregon. This field grows consecutively winter wheat with oats as the spring rotational crop. The field has a history of ACCase inhibitor herbicides applications. The *L. multiflorum* sub-populations from this winter wheat field survived applications of pinoxaden, clethodim, sethoxydim, clodinafop, tralkoxydim and fenoxaprop. The grass weed management in recent years in this field has been mainly an application of an ACCase inhibitor twice per year. The frequency of ACCase inhibitor herbicides use lead to the evolution of these *L. multiflorum* sub-populations with cross-resistance to APP, CHD and PPZ herbicides. Indeed, grass weeds have frequently exhibited cross-resistance to APP and CHD herbicides (SEEFELDT et al., 1994). ACCase inhibitor resistance is one of the most common modes of action reported in grass weed resistance (HEAP, 2011) and it has been established that only a few years of use are enough to build resistance. Previous reports have identified resistant biotypes after only few years of use of ACCase-inhibitor herbicides (HEAP and KNIGHT, 1986). This study determined that one of the *L. multiflorum* sub-populations is cross-resistant to four herbicides. Evaluation of resistance patterns is necessary to determine alternative herbicides that can be used to manage resistant populations (KUK et al., 2008). In this study, we tested three classes of ACCase-inhibitor herbicides, aryloxyphenoxypropionates (clodinafop), cyclohexanediones (clethodim and sethoxydim) and phenylpyrazolines (pinoxaden). Sethoxydim is not selective for cereal crops and is registered for grass control in dicotyledoneous crops (CAMPBELL and PENNER, 1985); however, two of the sub-populations tested (1 and 2) were resistant to this herbicide. Resistance to pinoxaden, clethodim and clodinafop could probably be due to target-site mutations. In most cases where the resistance mechanism has been determined, it is caused by to the presence of an insensitive form of ACCase caused by point mutations in the gene encoding the CT domain of plastidic ACCase (DEVINE, 1997). In a few cases, graminicide resistance has been shown to be caused by other mechanisms (HALL et al., 1997; MENEZES and DEPRADO, 1996; DEPRADO et al., 1999). For example, an insensitive ACCase has been identified as the mechanism of resistance in biotypes of *A. myosuroides* (COCKER et al., 1999), *A. fatua* (SEEFELDT et al., 1996), *Avena sterilis* (MANEECHOTE et al., 1994), *Digitaria ischaemum* (KUK et al., 1999), *Eleusine indica* (LEACH et al., 1995), *L. multiflorum* (GRONWALD et al., 1992), *L. rigidum* (TARDIF et al., 1993), *Phalaris minor* (TAL et al., 1996), *Setaria faberi* (SHUKLA et al., 1997), *Setaria viridis* (MARLES et al., 1993) and *Sorghum halepense* (MARLES et al., 1993). A new chemical family PPZ was released in 2006 for grass weed control. The PPZ herbicide pinoxaden was used in the wheat field where the tested sub-populations were collected. This indicates that cross-resistance to this herbicide evolved within a short period (less

than 5 years). The broad use of imidazolinone-resistant wheat varieties in Oregon highlights the need of determining multiple-resistance patterns of *L. multiflorum* to ALS and ACCase inhibitor herbicides. This would be important to facilitate an integrated system for management of these resistant weed populations. Ongoing studies are taking place to elucidate the cross-resistance mechanism to ACCase-inhibitor herbicides in these sub-populations, exploring the target site-based mechanism.

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