$Ile_{2041}Asn$ substitution confers ACCase inhibitor resistance in foxtail barley (*Hordeum jubatum*)

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Introduction

Foxtail barley (*Hordeum jubatum* L.) is a perennial grass weed that is native to western North America and found throughout Canada[1]. As a facultative halophyte, foxtail barley is commonly present in saline areas of western Canada, where it grows best on wet, fertile and non-alkaline soils[1]. Few herbicides registered for foxtail barley control[2] can cause overreliance on these products and greater selection pressure for herbicide resistance. However, herbicide-resistant foxtail barley has not been documented globally to-date[3].

In 2022, lack of control of foxtail barley was observed following quizalofop treatment in three creeping red fescue (*Festuca rubra* L.) fields in the Peace Lowland ecoregion of northern Alberta, Canada (**Fig. 1**). ***The objectives of this research were to:*** (***a***) determine whether these foxtail barley populations were resistant to the acetyl-CoA carboxylase (ACCase)-inhibiting herbicides quizalofop or clethodim, and if so (***b***) determine the mechanism conferring ACCase inhibitor resistance.

Figure 1. Map of Canada showing the location of the Peace Lowland ecoregion where the putative ACCase inhibitor-resistant foxtail barley samples were collected.

Materials and Methods

Plant material

- >2000 seeds harvested from ≥20 putative-resistant plants that survived quizalofop in the three fields (PR-1; PR-2; PR-3) and two untreated fields with susceptible foxtail barley (S-1; S-2)

Single-dose screening

- Each accession was planted in 24×24 cm greenhouse flats filled with soil-less potting mixture
  - 20/18°C, 16 h photoperiod, 100 µmol m^{-2} s^{-1} supplemental light
- Plants (2-leaf stage) were treated with quizalofop (Assure® II, AMVAC) at 70 g ai ha^{-1} and Merge® surfactant (BASF) at 1% v/v
  - Moving-nozzle cabinet sprayer, TeeJet® 8002VS nozzle, 200 L ha^{-1} solution
- Plant survival evaluated 21 days after treatment (DAT)

Dose-response experiment

- Separate experiments for quizalofop and clethodim
  - Randomized complete block design with 4 replicates and 2 runs
- 8 foxtail barley plants (1-leaf stage) transplanted into 10×10 cm pots
- Herbicide treatment methodology as described above
  - Quizalofop rates: 0, 3, 6, 12, 24, 48, 96, 192, 384, 768, 1536 & 3072 g ai ha^{-1}
    - Assure® II with Merge® surfactant at 1% v/v
  - Clethodim rates: 0, 2.8, 5.6, 11.3, 22.5, 45, 90, 180, 360, 720, 1440 & 2880 g ai ha^{-1}
    - Centurion® with Amigo® adjuvant (BASF) at 0.5% v/v
- Plant biomass dry weight (DW) determined 21 DAT
- Data analyzed using 4-parameter Weibull type 1 model (quizalofop; **Eq. 1**) and Brian-Cousens hormesis model (clethodim; **Eq. 2**) in 'drc' package[4] of R v. 4.3.1[5]

ACCase gene sequencing

- Plastid ACCase region was amplified from DNA of survived resistant and untreated susceptible plants using universal primers[6] and amplified products were gel purified
- Sanger sequencing followed by sequence analysis using Geneious Prime software
- rhAmp genotyping assay developed for rapid detection of resistance

Results and Discussion

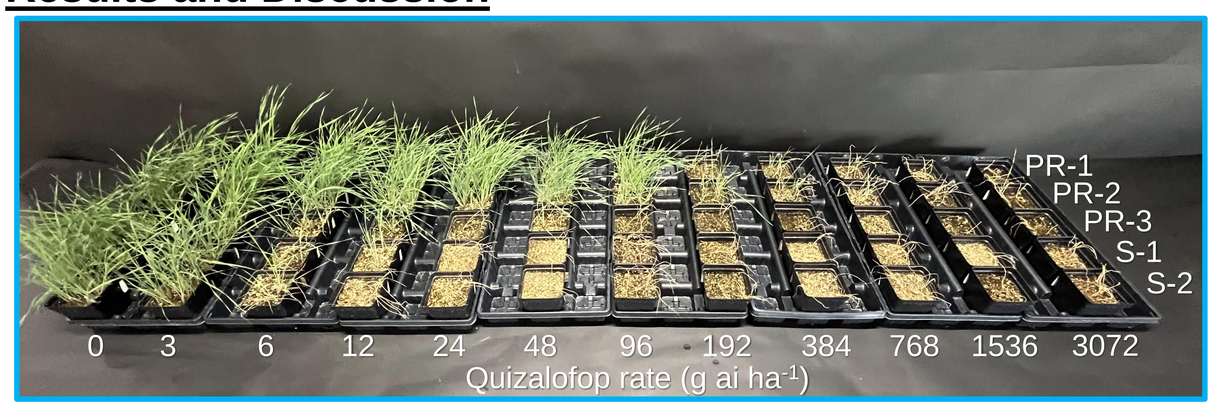

Figure 2. One replicate of the dose-response experiment showing the response of three putative acetyl-CoA carboxylase inhibitor-resistant foxtail barley accessions (PR-1; PR-2; PR-3) and two susceptible control accessions (S-1; S-2) 21 days after treatment with 12 rates of quizalofop.

Conclusions

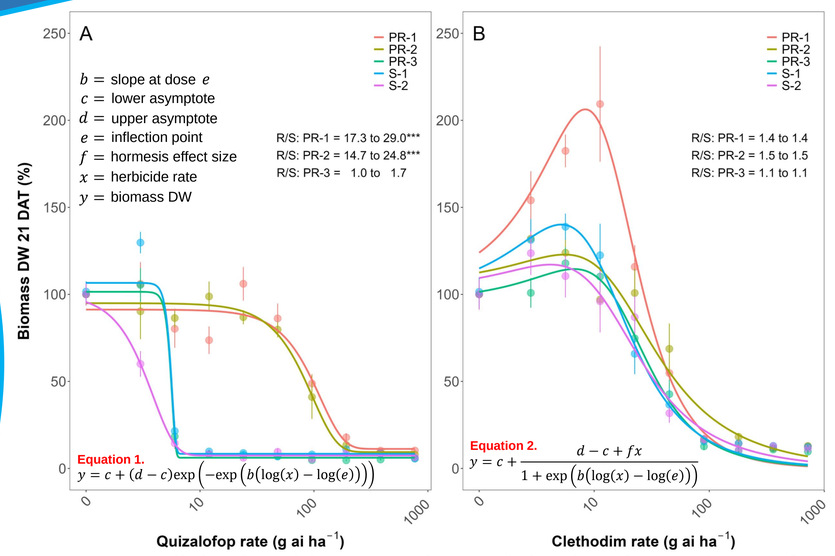

Figure 3. Foxtail barley biomass dry weight (DW) 21 days after treatment (DAT) with (**A**) quizalofop and (**B**) clethodim. Dots indicate means; bars indicate ±SE. *** indicates difference between herbicide rates causing 50% biomass reduction (GR_{50}) at *P <0.0001*.

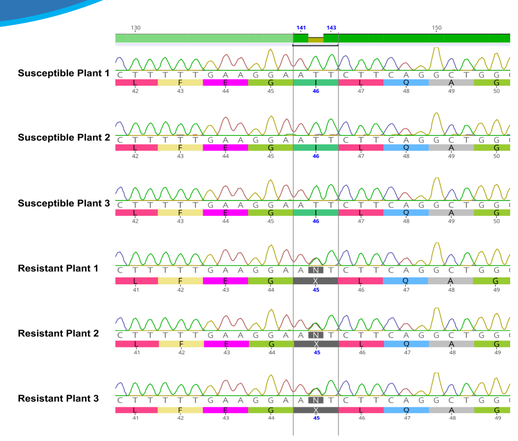

Figure 4. Partial sequence alignment of the plastid ACCase gene of ACCase inhibitor-resistant (lower) and -susceptible (upper) foxtail barley plants. The region between the vertical lines indicates a target site mutation resulting an $Ile_{2041}Asn$ amino acid substitution.

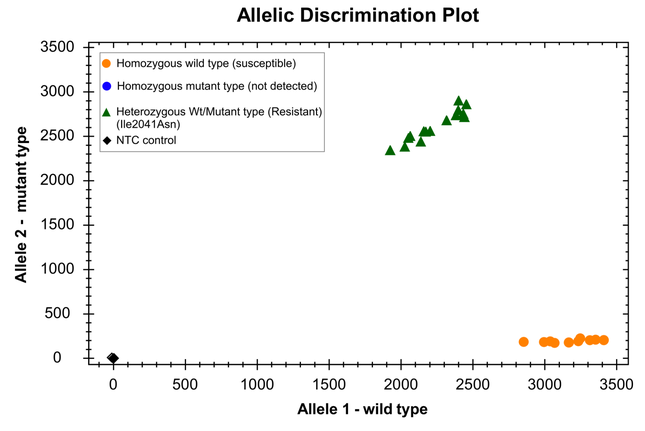

Figure 5. Allelic discrimination plot for the rapid rhAmp single nucleotide polymorphism (SNP) genotyping assay. The assay uses real time polymerase chain reaction for detection of the $Ile_{2041}Asn$ mutation in ACCase inhibitor-resistant foxtail barley plants.

- 79% (PR-1), 90% (PR-2) & 4% (PR-3) survival of quizalofop (70 g ai ha^{-1}) 21 DAT
- PR-1 exhibited 17.3- to 29.0-fold resistance, PR-2 exhibited 14.7- to 24.8-fold resistance and PR-3 was susceptible (R/S ≤1.7) to quizalofop (**Figs. 2 & 3A**)
- All three accessions were susceptible to clethodim (R/S ≤1.5) (**Fig. 3B**)
- PR-1 and PR-2 were heterozygous for a target site mutation resulting in an amino acid substitution at position $Ile_{2041}Asn$ (**Figs. 4 & 5**) shown to confer ACCase inhibitor resistance in other species[7]

Acknowledgments

Agriculture and Agri-Food Canada
Agriculture et Agroalimentaire Canada
Manitoba Crop Alliance
WGRF Advancing Agriculture through Research
RDAR Results Driven Agriculture Research
Government of Saskatchewan
Manitoba
Manitoba Pulse & Soybean Growers
Manitoba Canola Growers Association
Alberta Grains

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