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Acetyl-CoA carboxylase overexpression in herbicide resistant crabgrass (*Digitaria sanguinalis*)

Laforest, M.* , Soufiane, B.*, Page, E.R.§, Simard, M.-J.*, Obeid, K. ¤ & Nurse, R.E.§

Agriculture and Agri-Food Canada: *430 Gouin blvd, St-Jean-sur-Richelieu, QC, Ca; §2585 County Road 20, Harrow, ON, Ca; ¤OMAFRA, Harrow, ON, Ca

Abstract

Growers have to cope with increasing cases of herbicide resistant weeds. The development of genetic tests is helping the identification of resistance for specific species and is leveraging years of research to identify causal mutations reported in the literature. Whereas most of these studies describe target site mutations, other cases exist in nature where this molecular mechanism is not involved. The molecular descriptions of these cases are likely under-reported due to analytic challenges. A crabgrass biotype (*Digitaria sanguinalis*) from Southern Ontario tested positive for resistance to Acetyl-CoA Carboxylase (ACCase) inhibitors (WSSA group 1) herbicides (up to 4X the labelled rate) although none of the target site mutations previously known to confer resistance were detected. Our goal was to evaluate, using RNASeq, if any gene showed differential expression that could explain herbicide resistance. Both RNASeq results and confirmation by Reverse-Transcriptase Quantitative PCR (QRT-PCR) indicate an increase in the level of expression of the ACCase gene. The number of transcripts was 3.4 to 9.3 times higher in the resistant biotype compared to the susceptible population. The high variability of ACCase transcript levels in the resistant plants could be indicative of a genomic architecture promoting higher expression. The QRT-PCR assay developed could serve as a diagnostic tool when ACCase inhibitor resistance is suspected.

Material and methods

- Suspected group 1 resistant crabgrass plants were collected from a carrot/onion rotation in Leamington (ON, Canada) during the summer of 2015. They were subsequently grown to seed in a greenhouse at Harrow, ON.
- Dose response experiments were performed by treating susceptible and suspected resistant biotypes with 0x, 0.25x, 0.5x, 1x, 2x and 4 times the recommended dose for each of clethodim, fenoxaprop-p-ethyl, fluazifop-p-butyl, quizalofop-p-ethyl, and sethoxidim. Dry biomass was collected 28 days after treatment (DAT).
- Total RNA was extracted from leaf tissue using Qiagen RNeasy Plant Mini Kit.
- Sequencing libraries were prepared using the Illumina TruSeq Stranded Total RNA Library Prep Kit. Paired-end sequencing was performed at the Génome Québec Innovation Centre.
- EA-Utils[1] fastq-mcf was used to remove adaptor sequence and perform quality clipping. Trinity[2] *de novo* assembly was performed and comparisons of expression levels was done using Tophat2[3] and Cufflinks[4].
- ACCase expression was determined by qRT-PCR using β-actin as a reference gene. Each sample was subjected to a real-time PCR analysis in triplicates.
  - ACCase expression data were analyzed using the ΔΔCt method against the reference gene β-actin[5].

Discussion and Conclusion

- Resistance to group 1 herbicides was confirmed in an Ontario crabgrass biotype (Figure 1).
- No resistance conferring mutations were found in the ACCase gene (not shown).
- Plants were resistant to three Aryloxyphenoxypropionates and two cyclohexane-diones herbicides.
- Resistance level was 14 to 50 fold that of a susceptible biotype, depending on the active ingredient tested (Figure 2).
  - R factor = sethoxidim > fluazipfop-p-butyl > fenoxaprop-p-ethyl > quizalofop-p-ethyl > clethodim
- The transcriptome was assembled using Next Generation Sequencing technology.
- Using RNASeq, a transcript was identified to be constitutively overexpressed in both treated and untreated leaves.
  - **The overexpressed transcript coding for ACCase itself is what we expect to have caused the resistance.**
- Quantitative Reverse-Transcriptase PCR confirmed overexpression in other individuals of the resistant biotype population (Figure 3A).
- The level of overexpression of the ACCase gene varies amongst individuals of the resistant biotype population (i.e, from 3.4 to 9.3 fold) (Figure 3B).
  - Investigations are underway to understand the molecular mechanism(s) governing the observed overexpression.

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References

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Results

Figure 1. Herbicide susceptible and resistant crabgrass 28 days after treatment with fluazifop-p-butyl at 0 and 4 times (1000 g a.i. ha^{-1}) application rate.

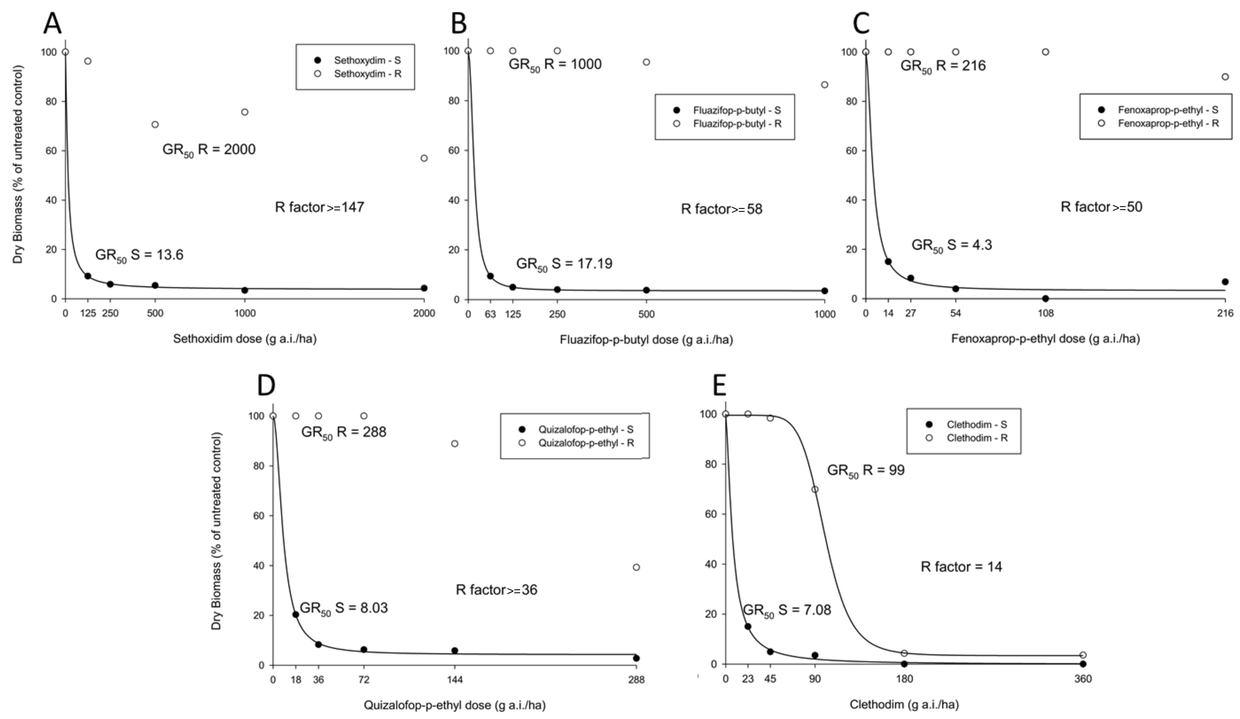

Figure 2. Dose-response curves for susceptible (dark points) and resistant (light points) crabgrass biotypes treated with 5 different group 1 herbicides (fenoxaprop-p-ethyl, sethoxidim, fluazipfop-p-butyl, quizalofop-p-ethyl and clethodim). Dry biomass was collected at 28 DAT. Log logistic equation: $Y=C + ((D-C)/(1+(x/GR_{50})^{b}))$; where C is the lower asymptote, D is the upper asymptote, b is the slope, and GR_{50} is the dose giving a 50% reduction in dry biomass or highest herbicide dose. R factor = GR_{50} R / GR_{50} S [6].

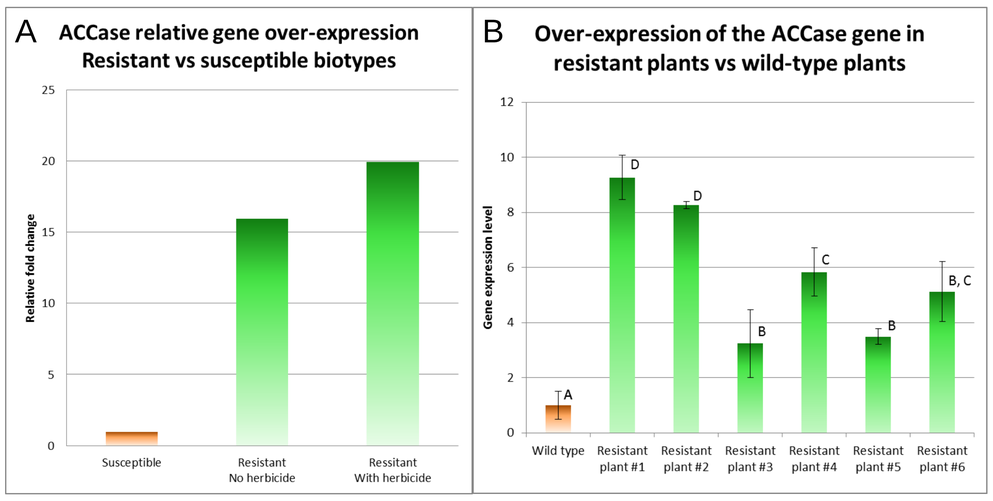

Figure 3. Abundance estimation of ACCase transcripts. A: Relative over-expression of the ACCase gene in the resistant vs susceptible biotypes with or without herbicide treatment as determined by RNAseq FPKM values. B: Over-expression of ACCase transcripts determined by Quantitative Reverse Transcriptase PCR ΔΔCt method. All values were normalized using actin expression measurements.