N-nitrosonornicotine reduction in dark tobacco varieties and smokeless product prototypes.

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2017

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Overview

- Background
  - Gene discovery and proof of concept
- Breeding of Stable Reduced Converter varieties
- NNN levels in LC and SRC dark tobacco varieties
- Expected impact of SRC varieties on NNN levels of DFC leaf crop
- NNN Levels in Smokeless Tobacco Prototypes with and without ZYVERT™ technology

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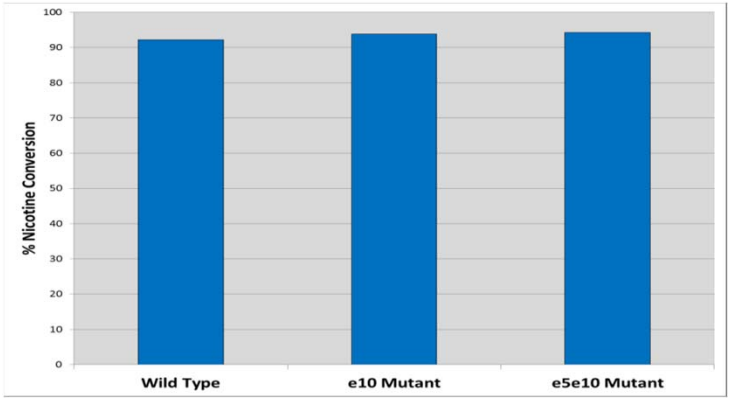
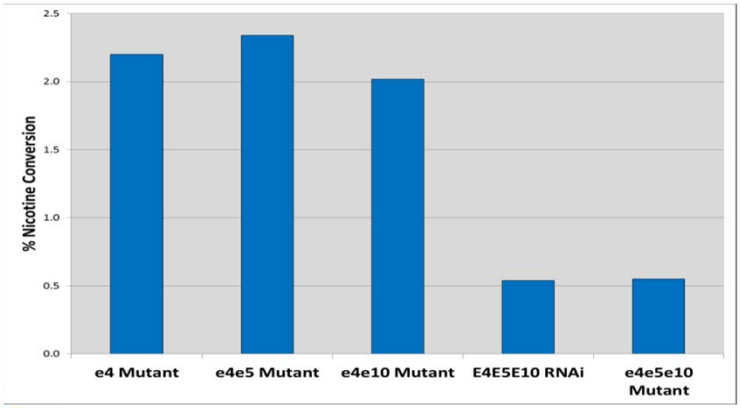
Background





Three nicotine demethylase genes mediate nornicotine biosynthesis in Nicotiana tabacum L.: Functional characterization of the CYP82E10 gene

Ramsey S. Lewis, Steven W. Bowen, Matthew R. Keogh, Ralph E. Dewey

Phytochemistry 71 (2010) 1988 - 1998

e4e4, e5e5, e10e10 = ZYVERT™ Technology

SRC Varieties = with ZYVERT™ Technology

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ALCS Breeding of SRC Varieties

SRC Varieties with ZYVERT™ Technology

- ALCS Proprietary Dark Tobacco Varieties
  - PD 7305 LC – PD 7305 SRC
  - PD 7309 LC – PD 7309 SRC
  - PD 7312 LC – PD 7312 SRC
  - PD 7318 LC – PD 7318 SRC
  - PD 7319 LC – PD 7319 SRC
- All five dark tobacco SRC varieties are eligible for seed certification
- Experimental Burley and Flue-cured SRC varieties were also developed

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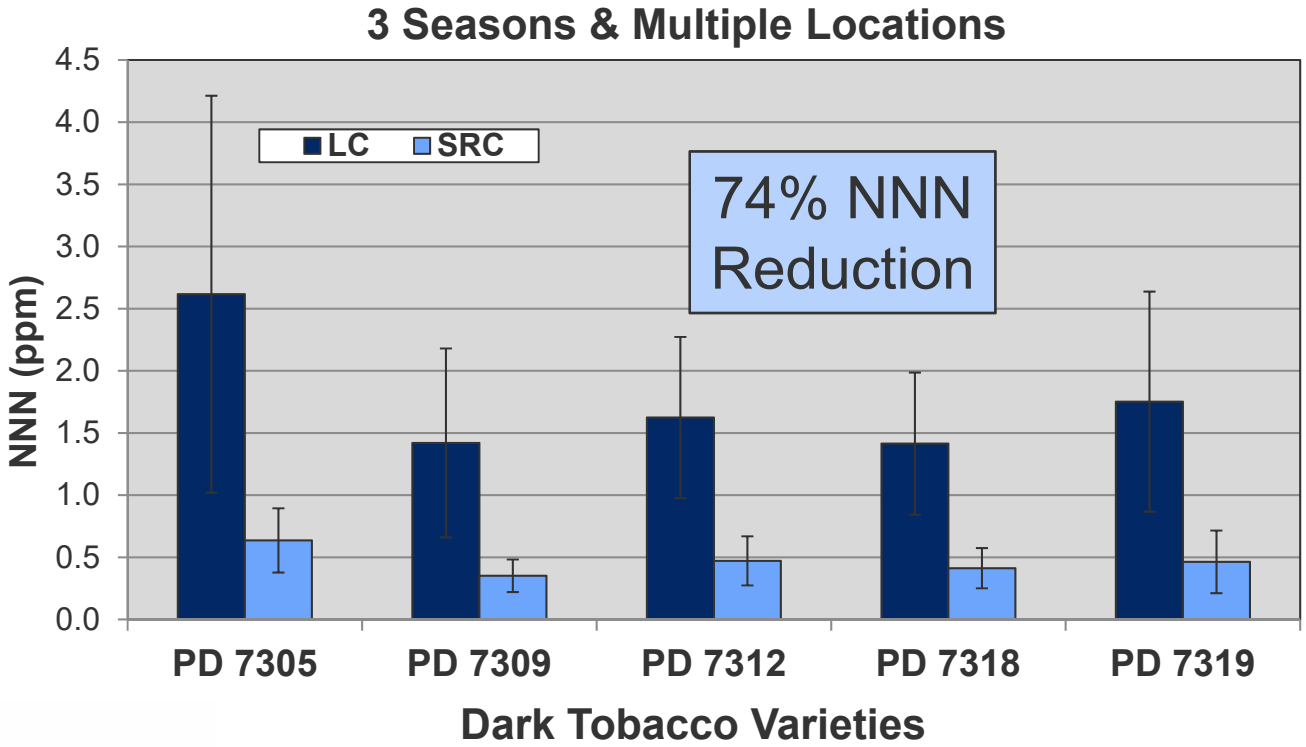
NNN Levels in LC and SRC Tobacco Varieties Grown in Research Plots



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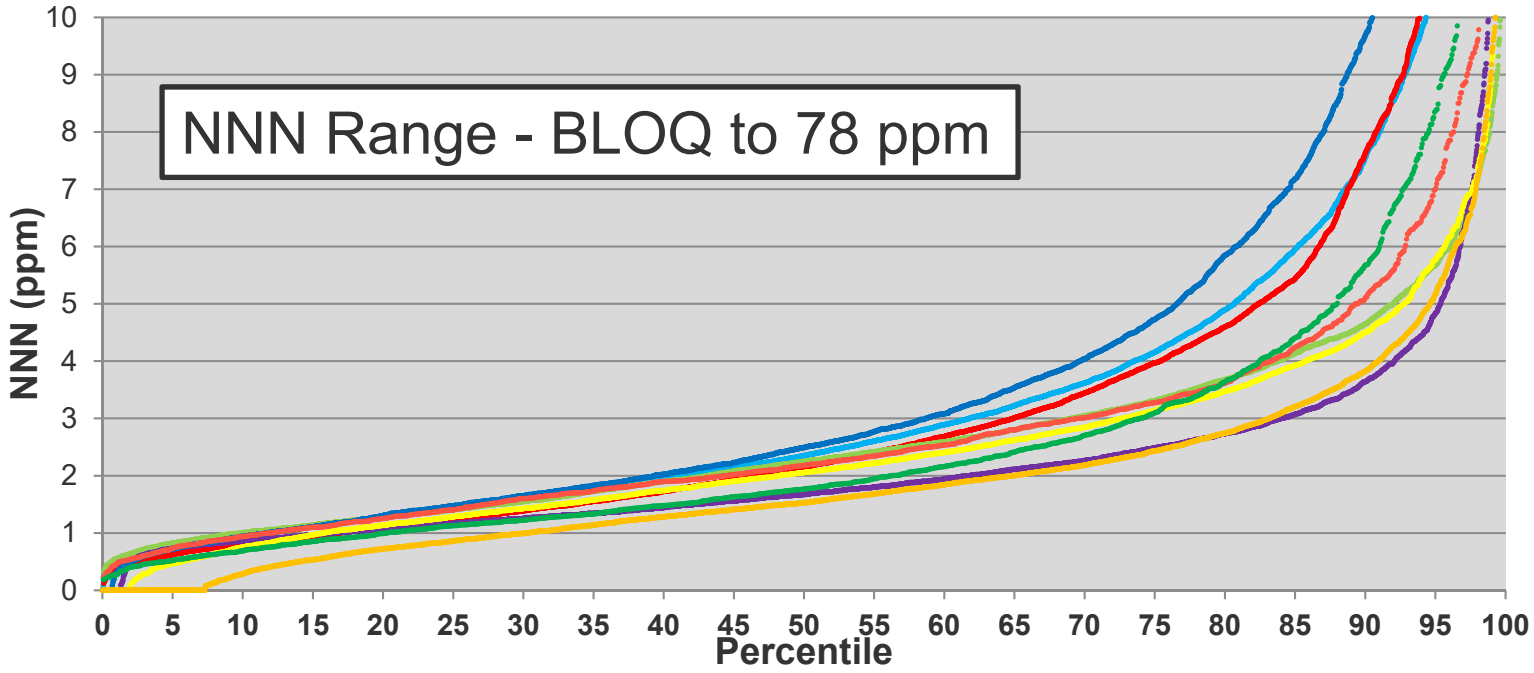
NNN Distribution in DFC Crop 05-13



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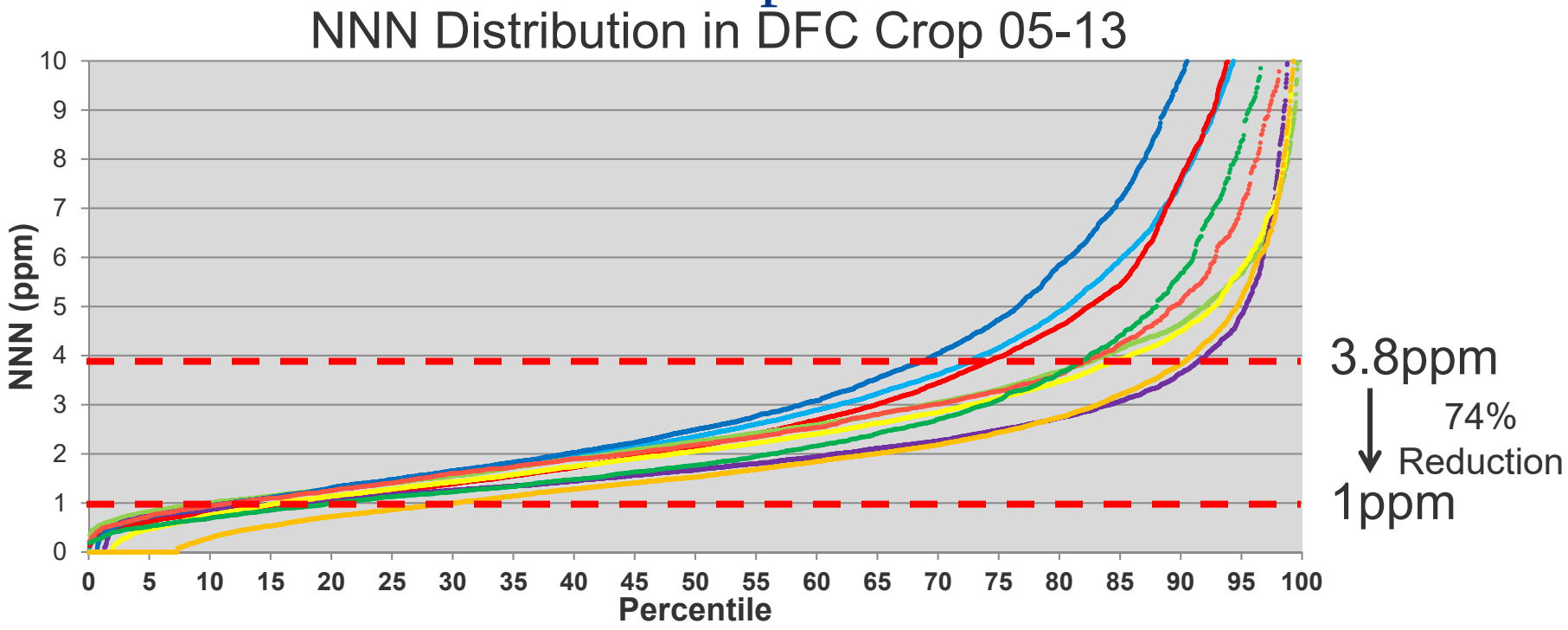
Expected Impact of SRC Varieties on NNN Levels of DFC Leaf Crop



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SRC Dark Tobacco Varieties Summary

- SRC dark tobacco varieties had NNN reduction of ~74% compared to LC varieties when grown in research plots.
- Between 2005 and 2013, depending on the year, 9% to 32% of the DFC tobacco crop had NNN levels below 1 ppm.
- Between 2005 and 2013, depending on the year, 68% to 91% of the DFC tobacco crop had NNN levels below 3.8 ppm.
- With the implementation of SRC varieties, depending on the year, 68% to 91% of the DFC tobacco crop could be below 1 ppm, with 9% to 32% in any given year being unusable.

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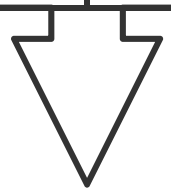
Smokeless Product Prototypes

Control	ZYVERT™
▪ 2014 regular production of LC varieties - Dark Fire-Cured - Dark Air-Cured - Burley Stems	▪ 2014 on-farm production SRC varieties with ZYVERT™ Technology - Dark Fire-Cured - Dark Air-Cured - Burley Stems



Aged for 2 years
Blended (dry flour)
Fermented (3 cures each)
Finished

Similar to CRP2 – Moist Snuff Reference Product

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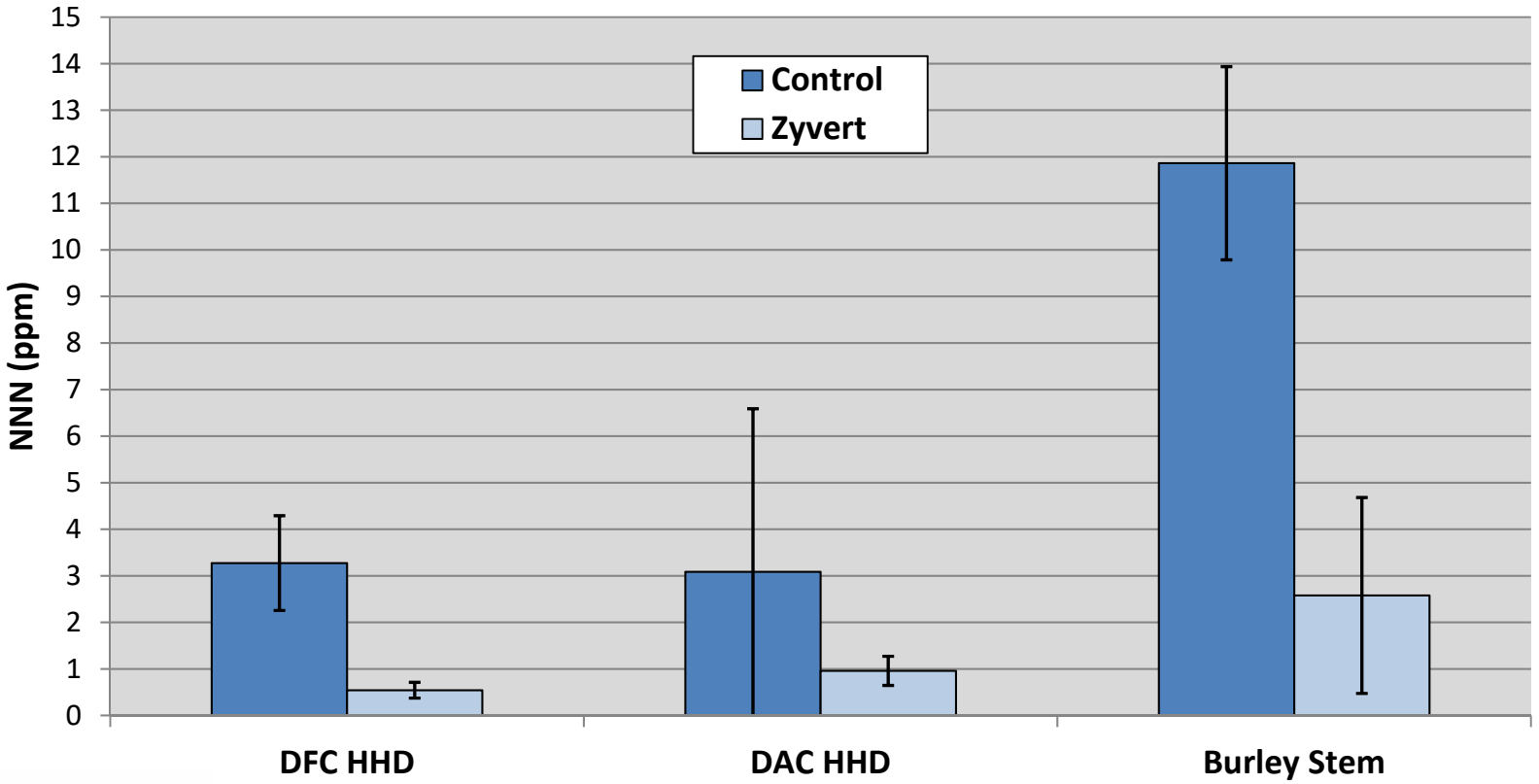
NNN Levels in Blend Components



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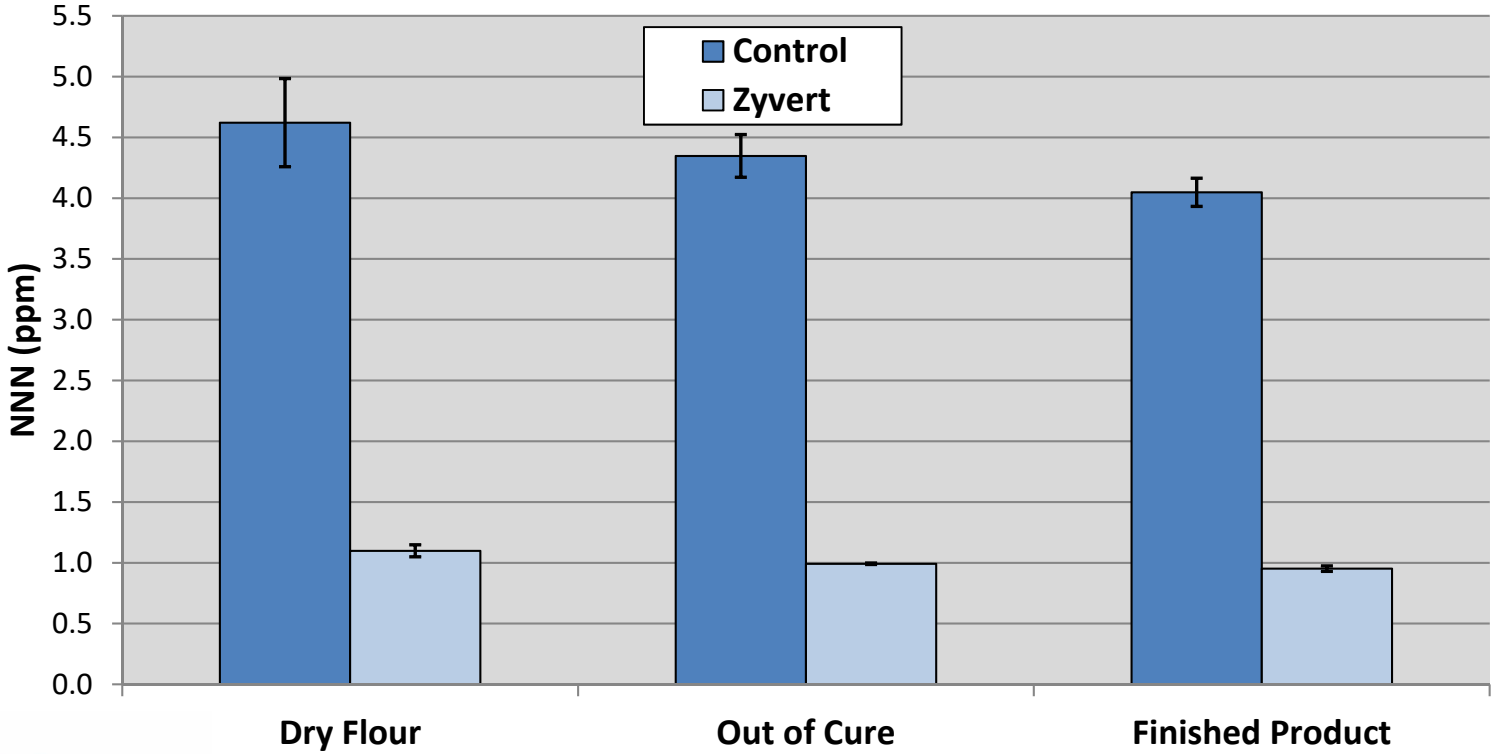
NNN Levels in DF, OOC & Finished Product Prototypes



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Smokeless Tobacco Prototype Summary

- A NNN reduction of 68% to 78% was observed in the individual blend components, Dry Flour and Finished products when SRC tobacco containing the ZYVERT™ technology was used to make smokeless tobacco prototypes.

- NNN levels in the finish product prototypes were comparable to the raw material.

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