Exploring Molecular Determinants of Invasion in Purple Loosestrife (*Lythrum salicaria*) using Metabolomics

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Roadmap

1. **Mechanisms of Invasion**
2. **Metabolomics**
3. **Exploring Molecular Determinants of Invasion in Purple Loosestrife**

Introduction to Purple Loosestrife (PLS)

- **Scientific Name:**
  Lythrum salicaria
- **Eurasian Origins**
- **Well-established by late 1800's**
- **Marsh and pasture perennial**

(Thompson *et al.*, 1987)

Metabolomics

- **Metabolites: Small molecules which are the products and intermediates of metabolism**
- **Metabolomics: Analysis of the complete set of a system's small molecules**
- **Snapshot of cellular/organismal physiology**
- **Allows for high-throughput analysis of biochemical phenotype**

(Reviewed by Fein, *et al.,* 2000)

Roadmap

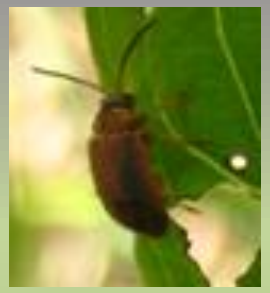
1 **Mechanisms of Invasion**

(Reviewed by Levine, J. M., *et al*. 2003. *Proc. R. Soc. Lond. B* 270:775; and Galatowitsch, *et al.* 1999. *Wetlands* 19:733)

2 **Metabolomics**

3 **Exploring Molecular Determinants of Invasion in PLS**

Environmental Mechanisms



Biotic

+

Abiotic

- **Favorable conditions enable increased biomass and seed production**

- **Decreased herbivory and competition allow rapid colonization**

- **Depends on phenotypic plasticity**

(Crawley, 1987; Bryant, *et al.*, 1988)

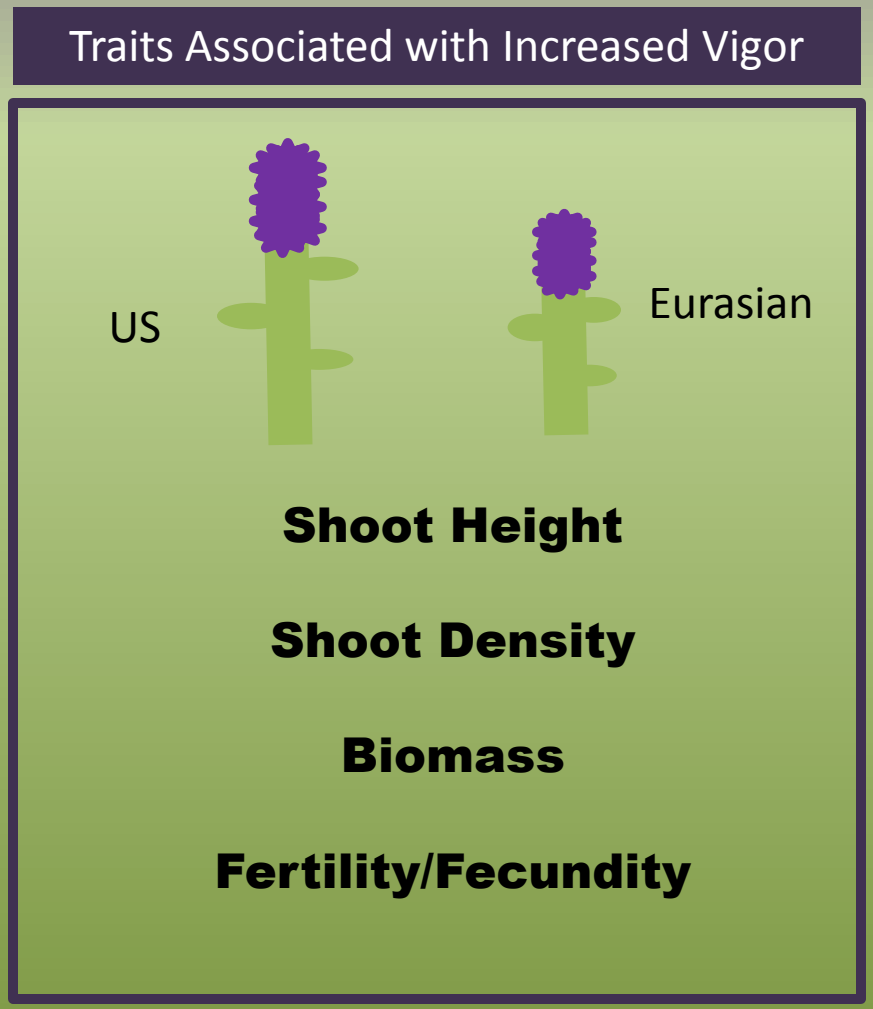
The Common Garden: An Uncommonly Good Idea

- **Grow plants from indigenous and non-indigenous populations in identical conditions**

- **Note phenotypic differences between the populations**

Same Conditions, Different Phenotypes…

- **Results: Environment is insufficient to explain increased vigor in US PLS**
- **Conclusion: ???**



(Blossey & Nötzold, 1995; Willis & Blossey, 1999; Edwards, *et al.,* 1998; Chun, *et al*., 2007)

Evolutionary Mechanisms I

Genotypic

Evolution of Increased Competitive Ability (EICA)

- **Absence of selective pressure of herbivory leads to resource reallocation to growth and reproduction**

- **Depends on: Evolutionary selection favoring growth over defense**

(Blossey & Nötzold, 1995)

Evolutionary Mechanisms II

Genotypic

Introgression

- **Introgressive hybridization of PLS (*L. salicaria*) with US native *Lythrum* species, such as winged loosestrife (*L. alatum*)**

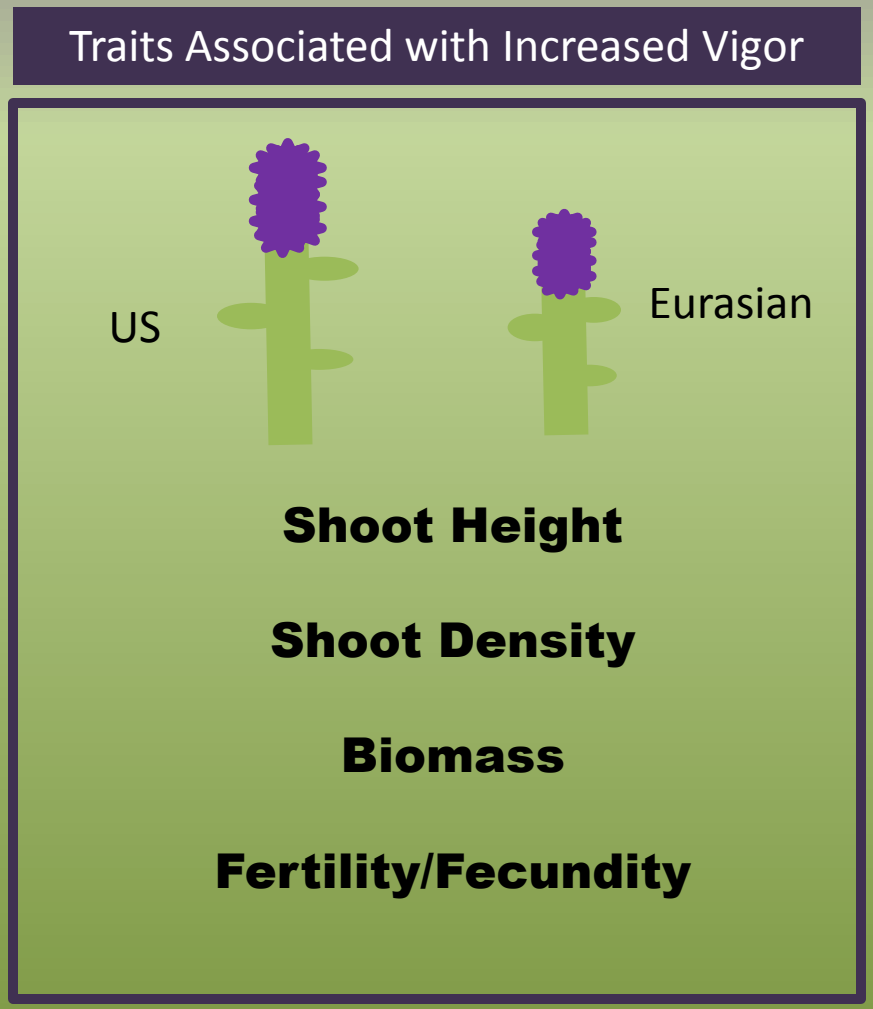
- **Depends on: Backcrossing of hybrid with parent species resulting novel phenotypes**

(Anderson, 1949; Anderson*, et al.,* 1995; Houghton-Thompson, *et al.,* 2005)

Same Conditions, Different Phenotypes…

Genotypic-Environmental Interaction

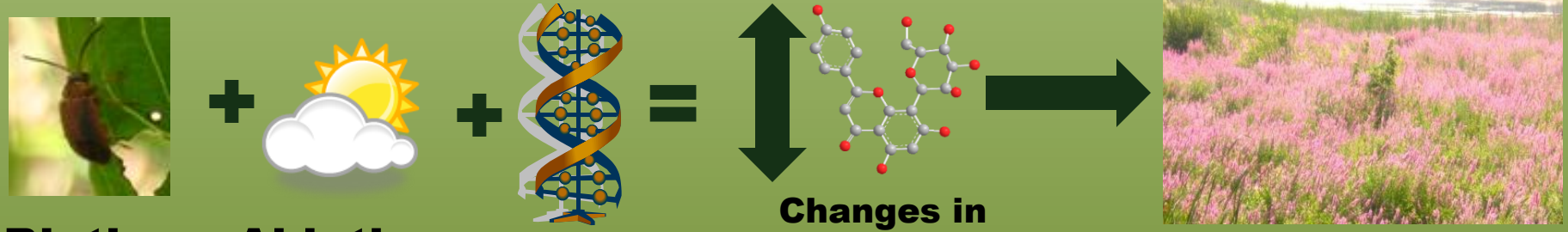
- **Results: Environment or genotype alone is insufficient to explain increased vigor in US PLS**
- **Conclusion: Genotypic contributions are influenced by environment**



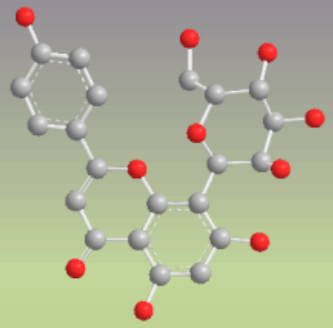
(Blossey & Nötzold, 1995; Willis & Blossey, 1999; Edwards, *et al.,* 1998; Chun, *et al*., 2007)

Predictions

- Selective pressures have caused US PLS populations to reallocate metabolic resources
- These metabolic changes have contributed increased vigor in US PLS
- These changes will result in significant differences between the secondary metabolite profiles of US and Eurasian PLS



Secondary Metabolites



What Are They?

- "Inessential" or group-specific small molecules
- Often involved in interactions such as defense or communication
- Common plant secondary metabolites:
  - Alkaloids, Terpenoids, and Phenols

Why Are We Studying Them?

- Involved in herbivore defense and allelopathy
- Usually relatively high abundance

(Reviewed by Bennet and Wallsgrove, 1994)

Roadmap

1 Mechanisms of Invasion

2 **Metabolomics: The study of a system's small molecules**

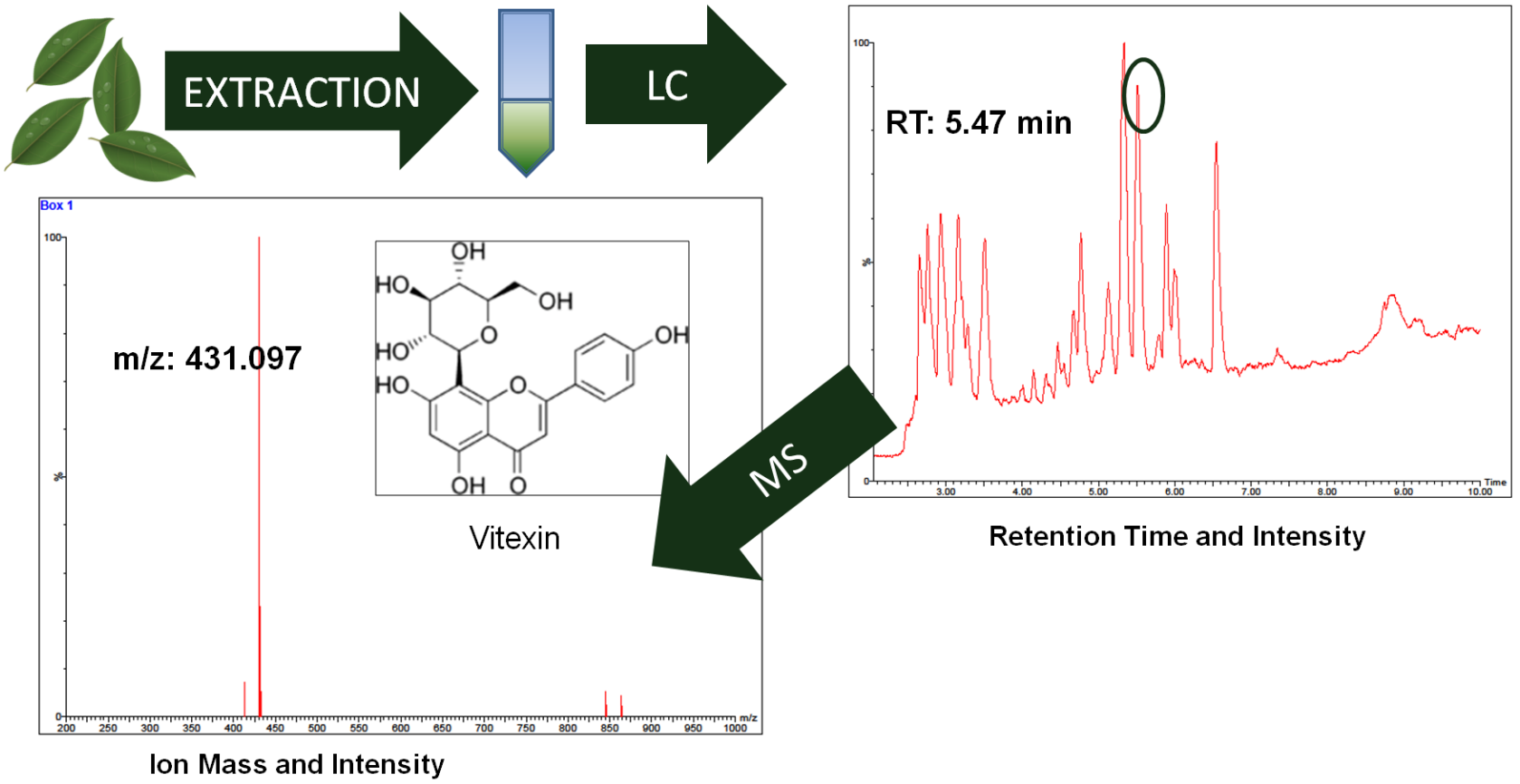
3 Exploring Molecular Determinants of Invasion in PLS

Metabolomics

- **Metabolites: Small molecules which are the products and intermediates of metabolism**
- **Metabolomics: Analysis of the complete set of a system's small molecules**
- **Snapshot of cellular/organismal physiology**
- **Allows for high-throughput analysis of biochemical phenotype**

(Reviewed by Fein, *et al.,* 2000)

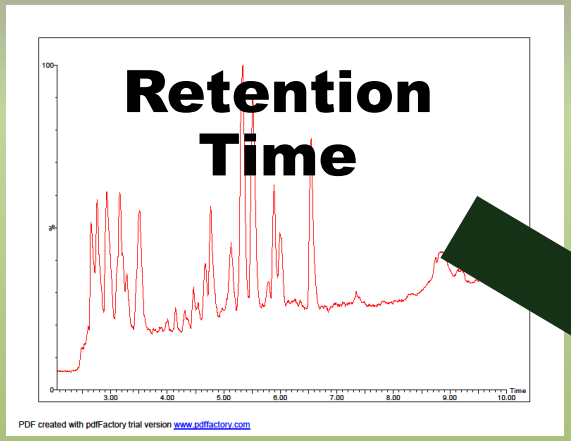
Liquid Chromatography-Mass Spectrometry (LC-MS)

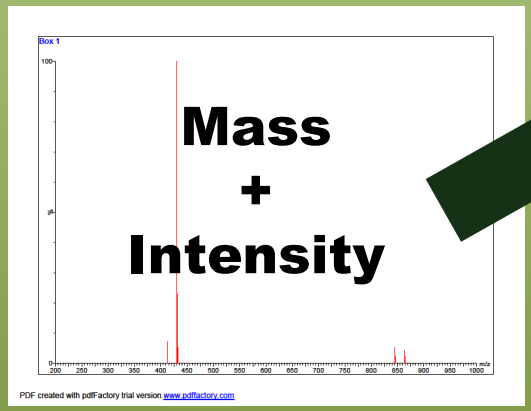


The mass data for this feature are consistent with VITEXIN, a compound previously identified in purple loosestrife

(Rauha, *et al.*, 2001)

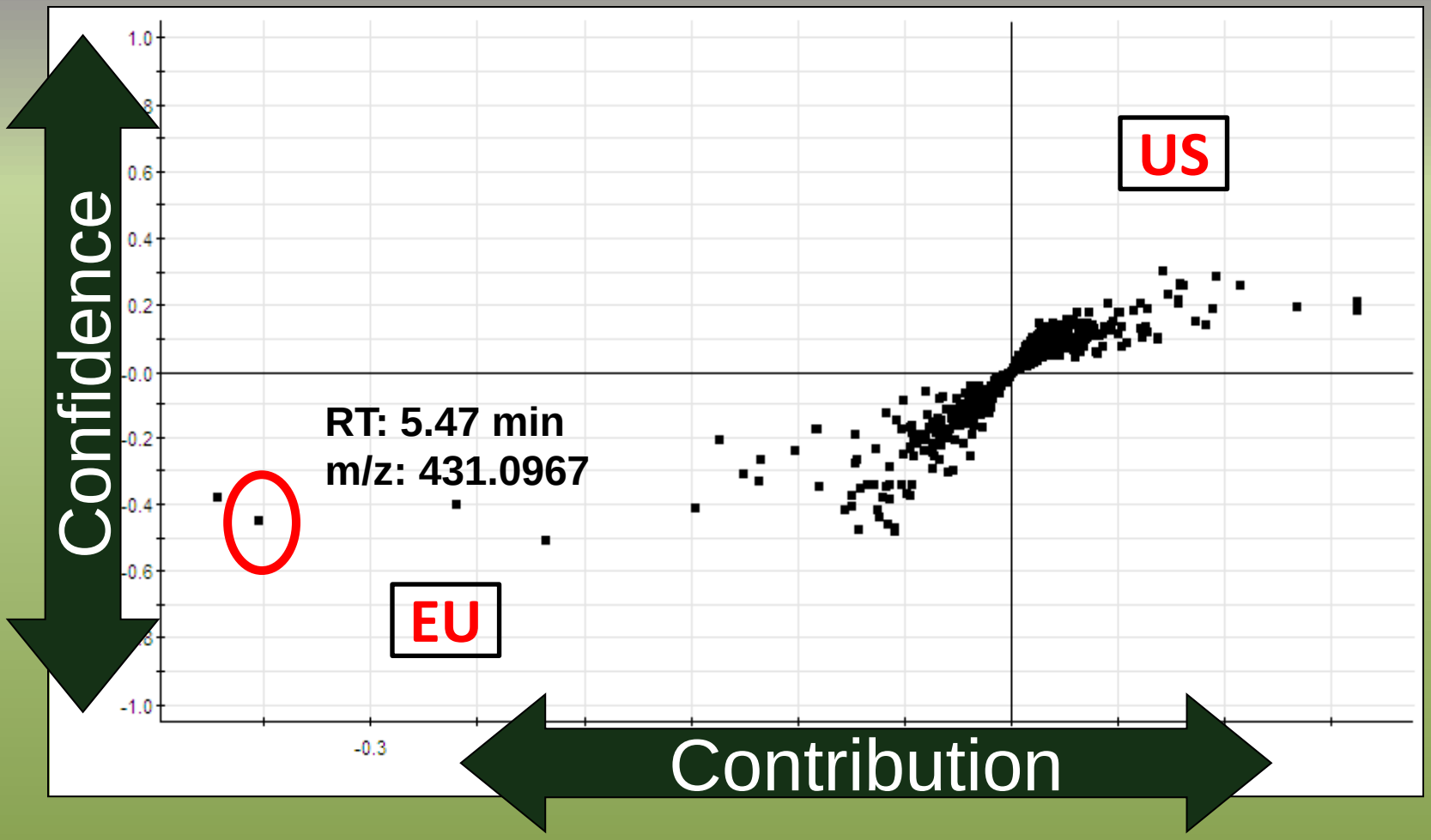
Multivariate Data Analysis (MDA)





Feature

S-Plot



(See Patterson, *et al.*, 2008. *Anal. Chem.* 80:665)

Roadmap

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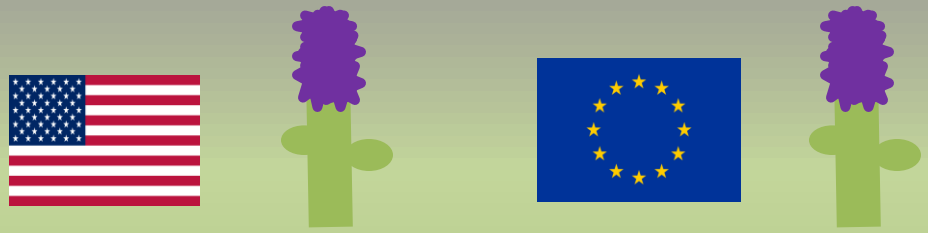
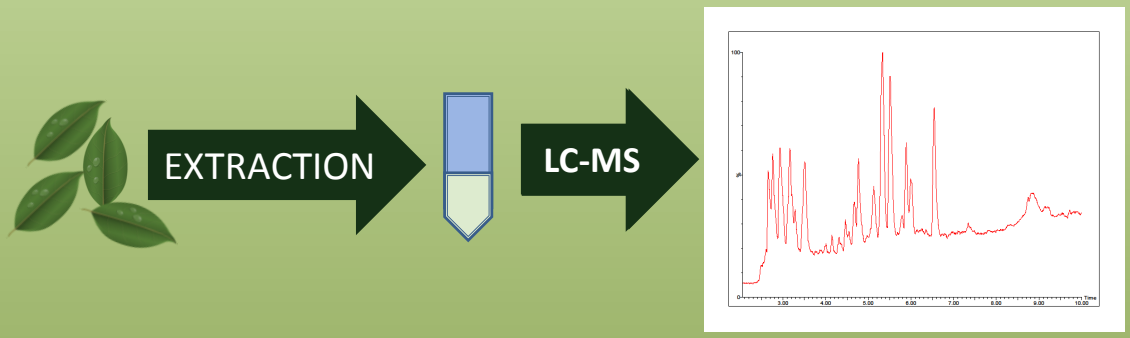
Progress

Grow US and European Plant in a Common Garden

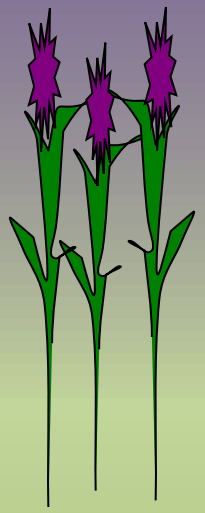


Develop metabolite profiles from extracts using LC-MS



Identify features in LC-MS data that contribute to origin-dependant variations in metabolite profiles.

Historically Low Herbivory (HLH):
Little, if any, beetle feeding

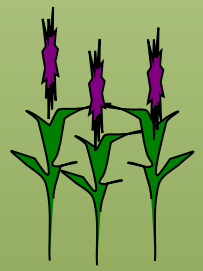


Invasion from Europe;
release from specialist
herbivores

~180 Years ago

1992

Introduction of
biocontrol agents



Native

Historically High Herbivory (HHH):
Often over 90% plant defoliation

?

(Adapted from Gina Quiram,2010)

Common Garden

Collection Site	Symbol	Population Type
Stará Hlína	CR	**Native**
Zliv	CR	**Native**
Hall's Marsh	MN	**HLH**
Pottery Pond	MN	**HLH**
Pig's Eye	MN	**HHH**
Circle Lake	MN	**HHH**

4 

Maternal Lines

x 8 

Plants

=

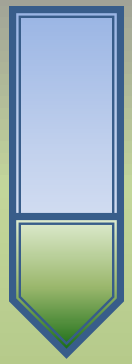
32 plants / Population

Harvest and Extraction

x 2

EXTRACTION

2

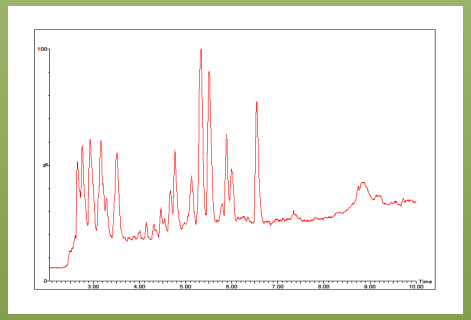


Replicate extracts/plant

LC-MS and MDA

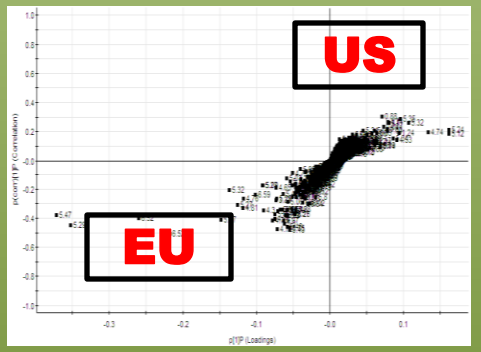
170 total extracts

LC-MS



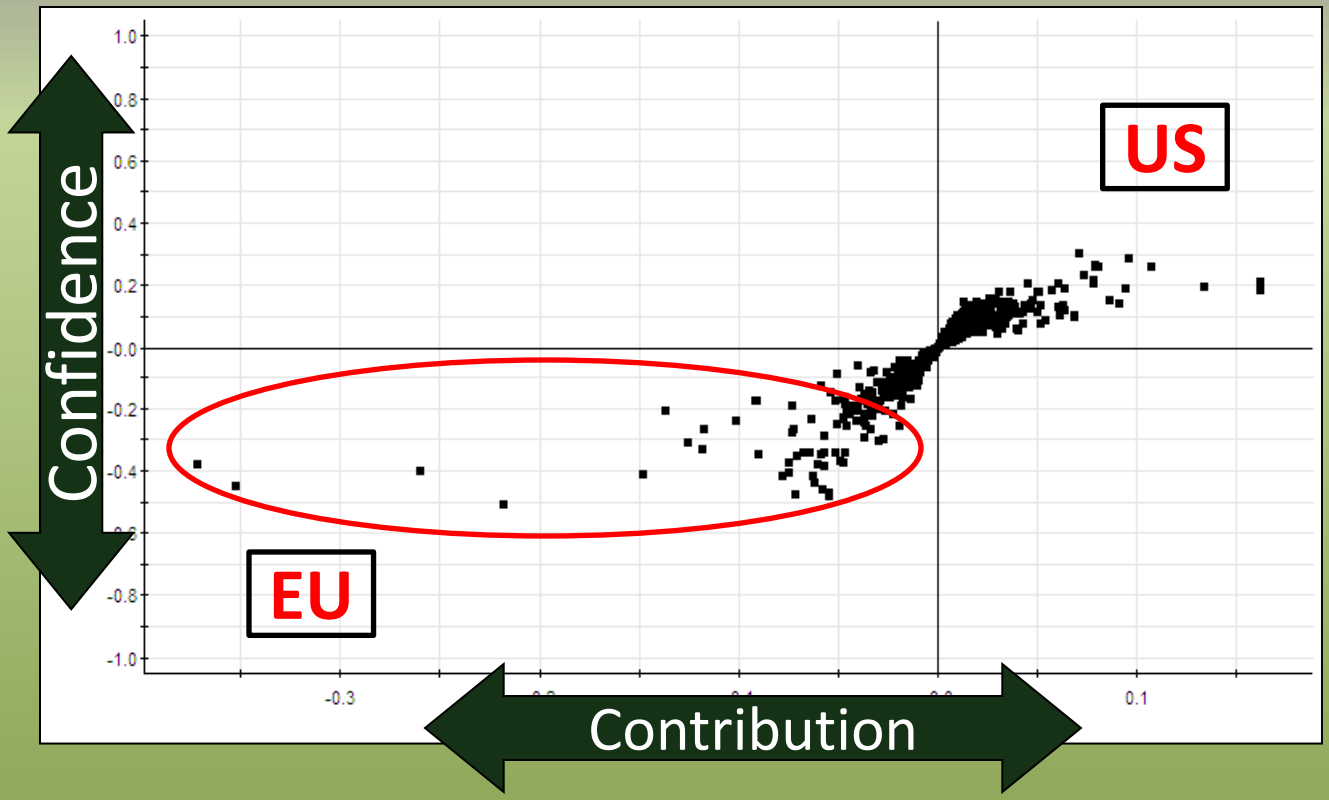
170 Metabolic Profiles

MDA



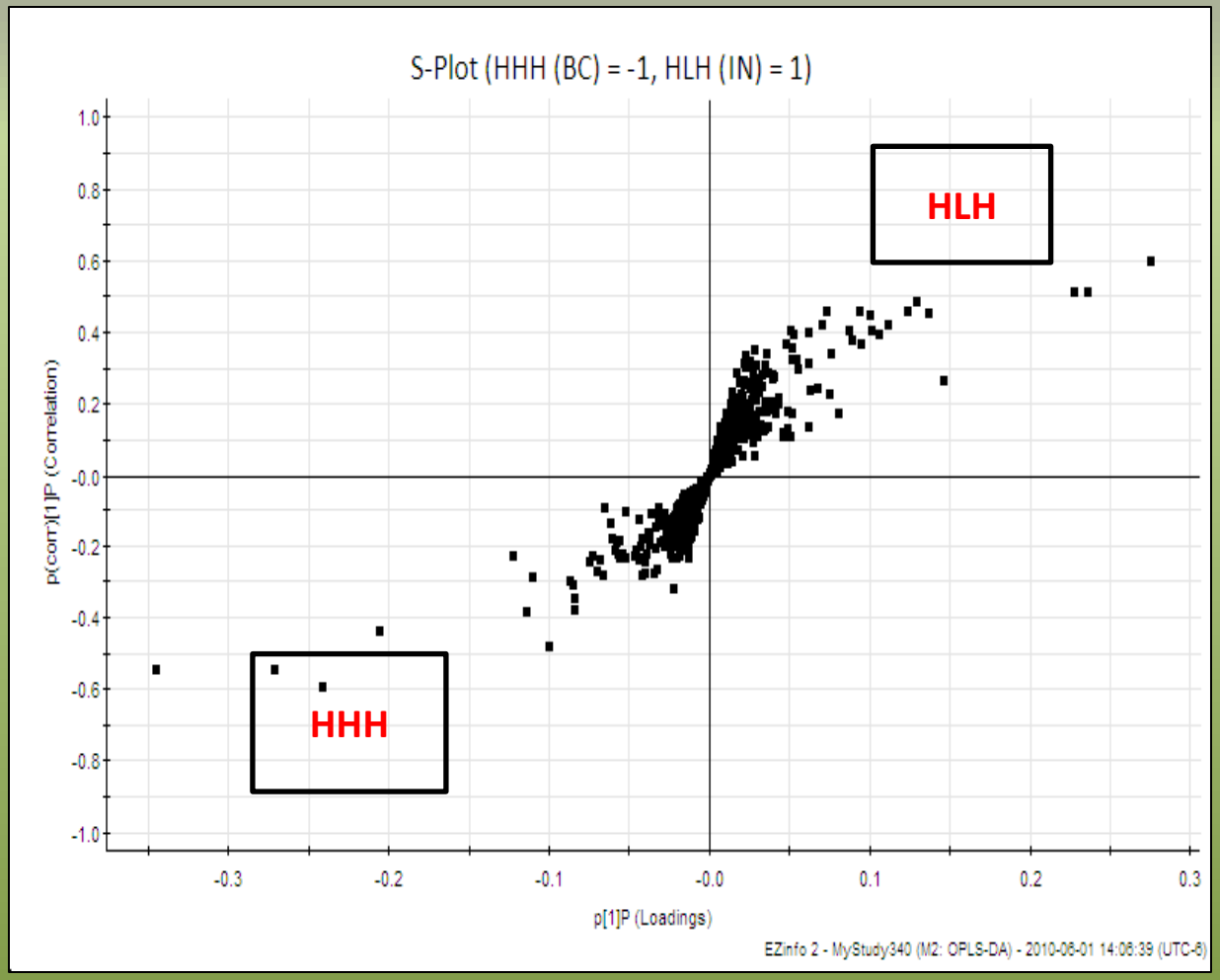
700 Features

EU Genotypes: Greater Overall Metabolic Diversity?



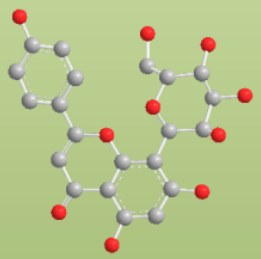
More EU features are contributing to separation than US features are contributing

What relationship exists between EU and HHH/HLH Features?



Future Directions

Purify and characterize the chemical structures of metabolites using preparative HPLC and NMR techniques



Test metabolites for bioactivity on *Gallerucella spp.* beetles

Allelopathy?

Implications

Future results could be used to inform invasive species management:

- **Better understand how PLS successfully invaded**
- **Block pathways of invasion**
- **Better predict whether a species is likely to become invasive**

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- **UMN College of Biological Sciences**

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