Breeding Pool and Market Class Differentiation in Leaf Physiology and Secondary Metabolism in Cultivated Sunflower

Chase Mason, Jordan Dowell
University of Central Florida
-
Alan Bowsher, Amna Jamshad, Rahul Shah,
John Burke, Lisa Donovan
University of Georgia

Jordan Dowell - PhD 2021 (UCF)
USDA NIFA Postdoctoral Fellow (UC Davis)
GWAS + phenotypic data analysis

Alan Bowsher, PhD – HPLC phenotyping
Amna Jamshad, MD – leaf phenotyping
Rahul Shah, MD – leaf phenotyping
Lisa Donovan, PhD – phenotyping + design advice
John Burke, PhD – GWAS + interpretation advice

Why focus on leaves?

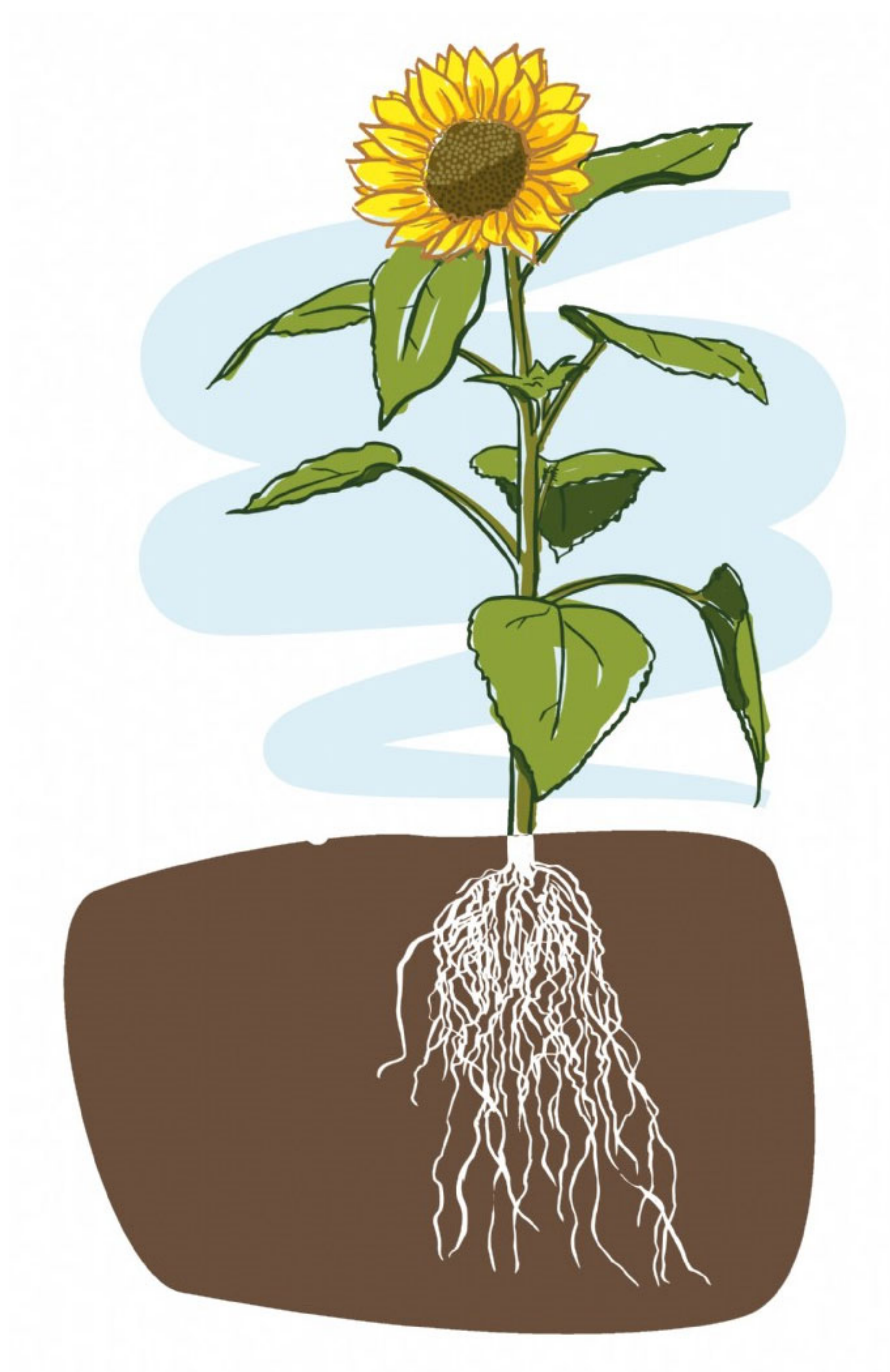
Leaf traits influence:

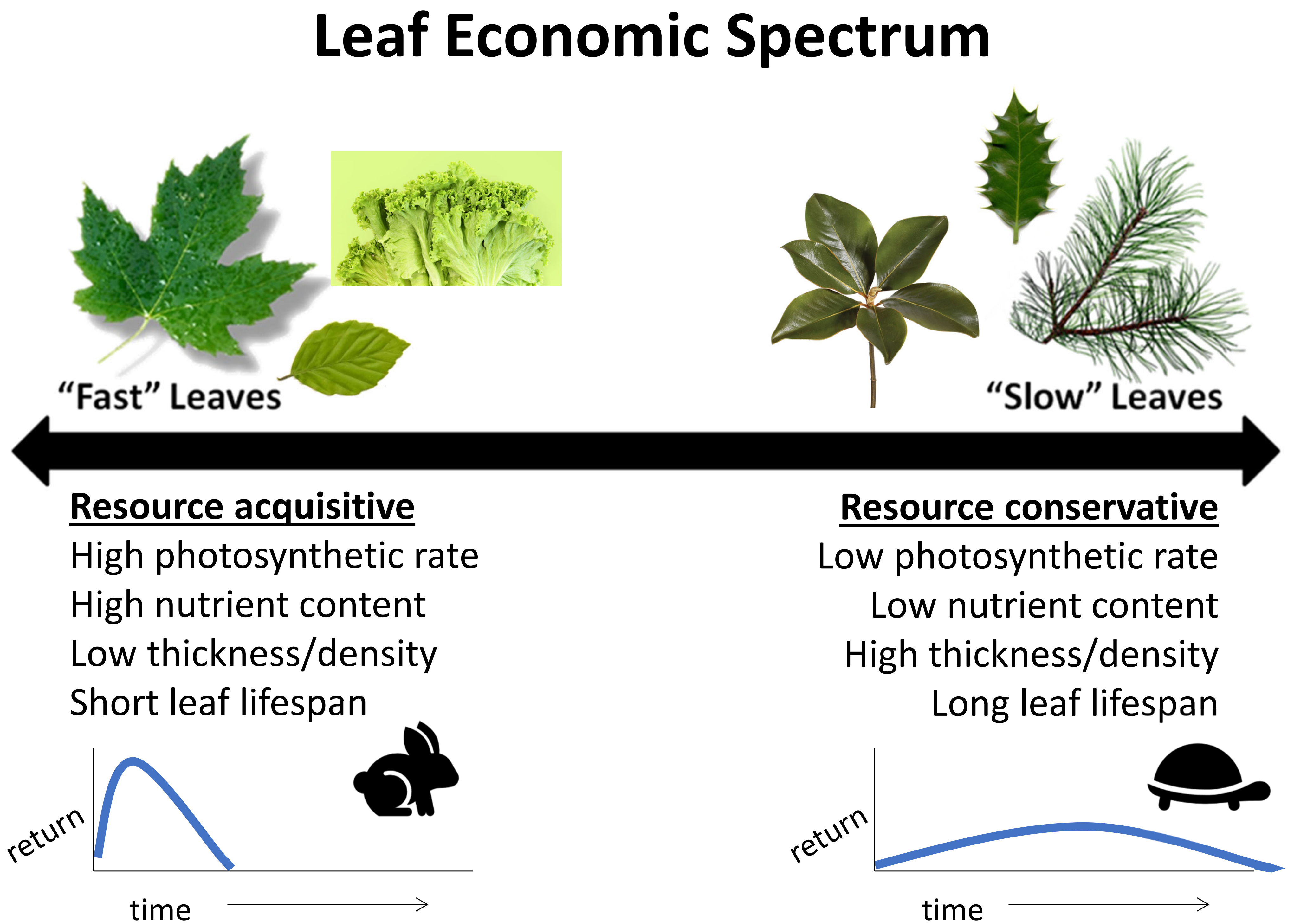
Assimilation (capture CO_2 + light)

Growth rate (product of leaf assimilation, leaf cost and lifespan, and plant allocation to leaves)

Abiotic stress tolerance (loss of water, nutrient resorption)

Biotic stress tolerance (herbivory + disease resistance)




Leaf Economic Spectrum
"Fast" Leaves
"Slow" Leaves
Resource acquisitive
High photosynthetic rate
High nutrient content
Low thickness/density
Short leaf lifespan
Resource conservative
Low photosynthetic rate
Low nutrient content
High thickness/density
Long leaf lifespan
return
time
return
time

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Research Article

Inter- and intraspecific variation in leaf economic traits in wheat and maize

Adam R. Martin[1,2*], Christine E. Hale[1], Bruno E. L. Cerabolini[3], Johannes H. C. Cornelissen[4], Joseph Craine[5], William A. Gough[1], Jens Kattge[6,7] and

Annals of Botany 123: 107–120, 2019
doi: 10.1093/aob/mcy147, available online at www.academic.oup.com/aob
ANNALS OF BOTANY Founded 1887

Intraspecific variation in soy across the leaf economics spectrum

Fallon J. Hayes[1], Serra W. Buchanan[1], Brent Coleman[2], Andrew M. Gordon[2], Peter B. Reich[3,4], Naresh V. Thevathasan[2], Ian J. Wright[5] and Adam R. Martin[1,6,*]

Functional Ecology
BRITISH ECOLOGICAL SOCIETY
Functional Ecology 2017, 31, 604–612 doi: 10.1111/1365-2435.12790

Intraspecific trait variation across multiple scales: the leaf economics spectrum in coffee

Adam R. Martin*,[1], Bruno Rapidel[2,3], Olivier Roupsard[2,4], Karel Van den Meersche[2,4], Elias de Melo Virginio Filho[2], Mirna Barrios[5] and Marney E. Isaac[1]

Journal of Experimental Botany, Vol. 69, No. 22 pp. 5599–5609, 2018
doi:10.1093/jxb/ery322 Advance Access publication 5 September 2018
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

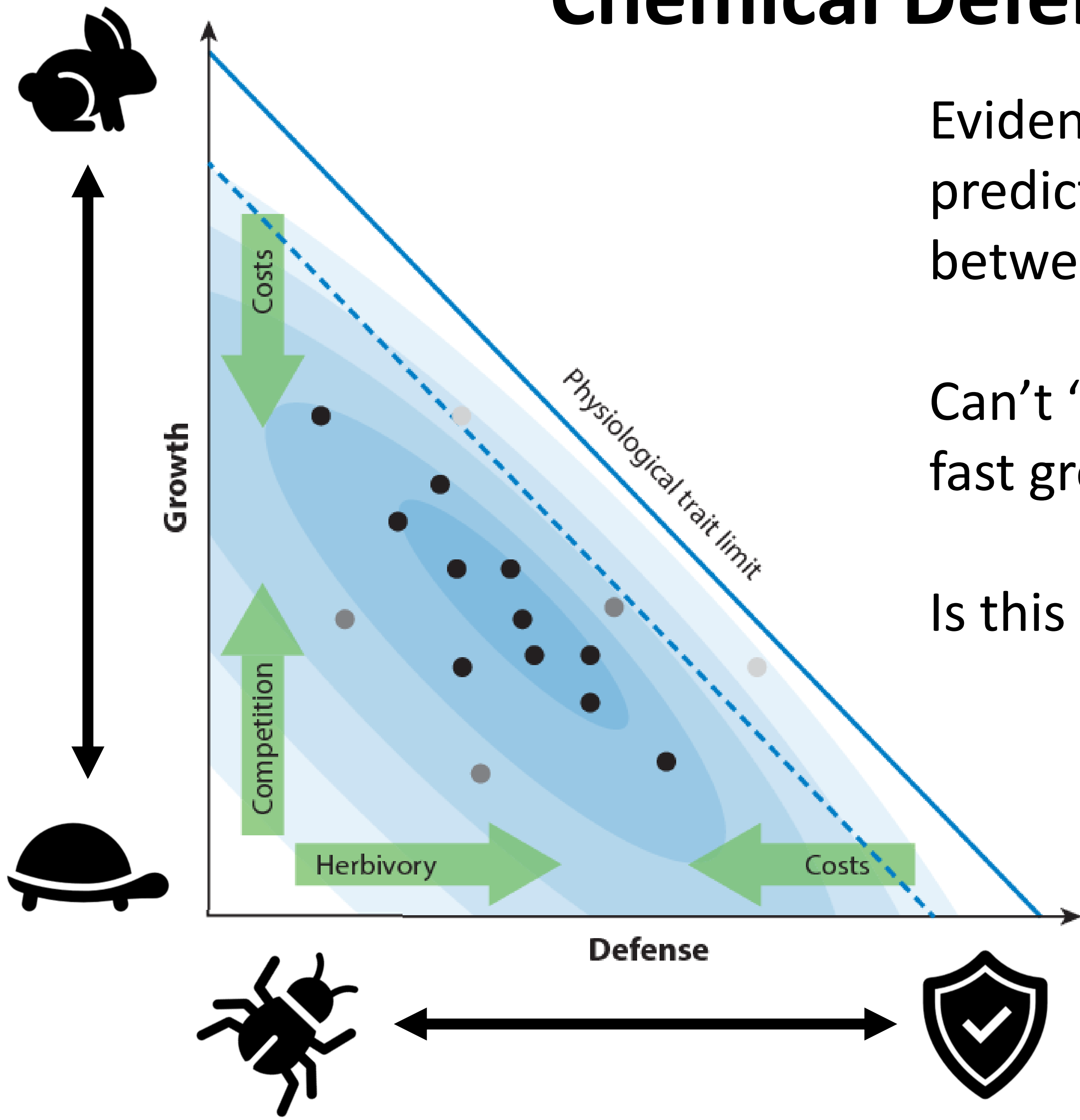
RESEARCH PAPER

Leaf economics spectrum in rice: leaf anatomical, biochemical, and physiological trait trade-offs

Dongliang Xiong[1,2,*] and Jaume Flexas[3]


Source: Kansas State
University Extension

Chemical Defense Investment



Evidence from wild plants predicts an inherent trade-off between growth and defense.

Can't "have it all" – don't see fast growth with high defense

Is this true within sunflower?

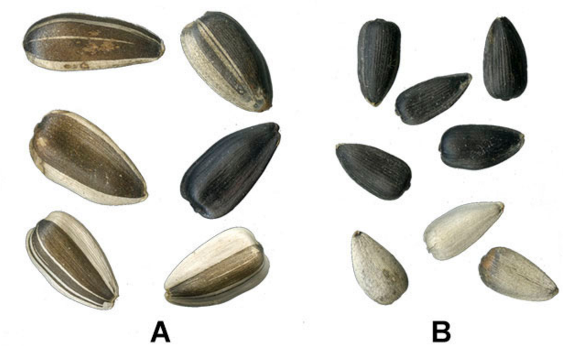
Zust T, Agrawal AA (2017) Trade-Offs Between Plant Growth and Defense Against Insect Herbivory: An Emerging Mechanistic Synthesis. Annual Review Plant Biology 68:513-534.

Market Classes + Breeding Groups

	Oilseed	NonOil
HA - maintainer	*HA Oil*	*HA NonOil*
RHA - restorer	*RHA Oil*	*RHA NonOil*
Others (OPVs, etc)	*Other Oil*	*Other NonOil*

USDA Agricultural Marketing Service
U.S. DEPARTMENT OF AGRICULTURE

SUNFLOWER SEED



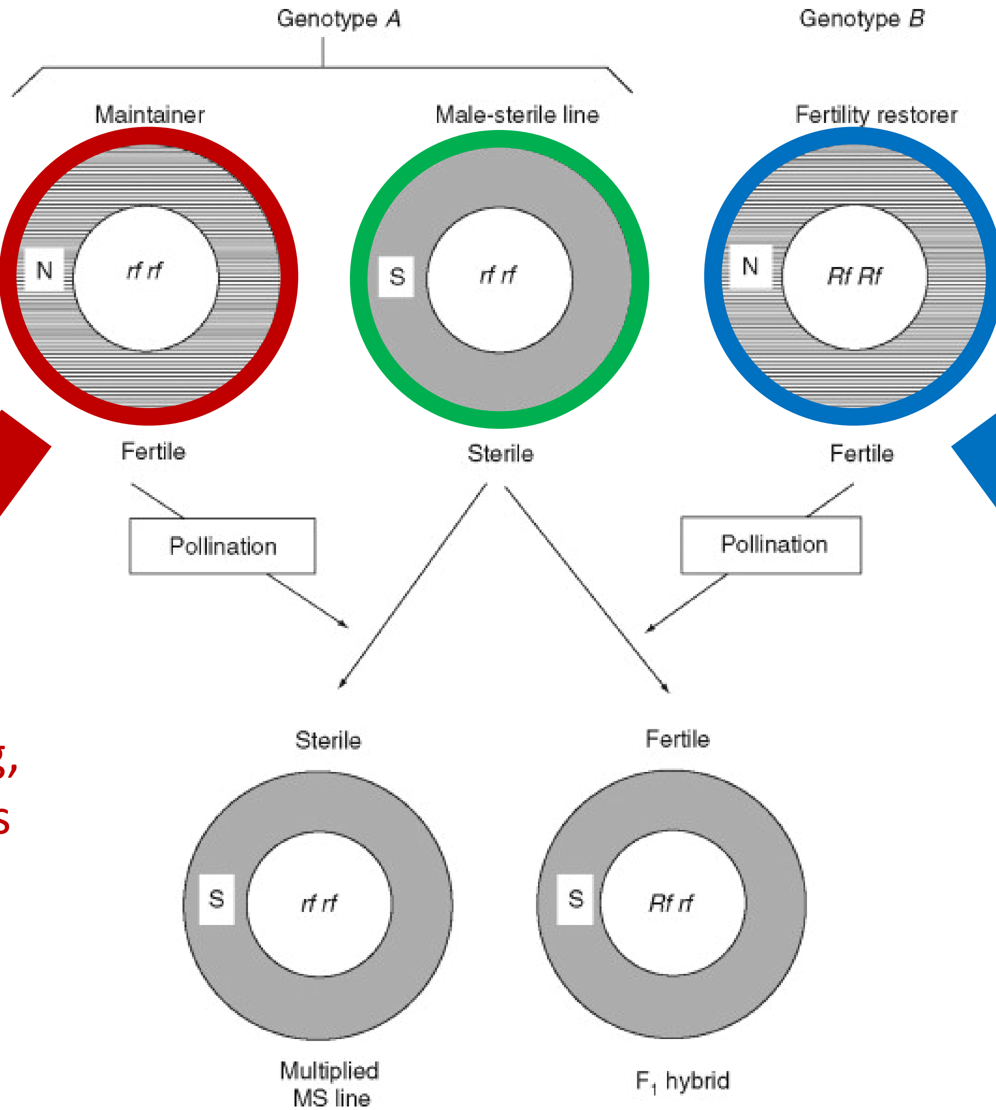
A. Confectionery Type. Black and white striped, thick-hulled, seed of the non-oil variety of sunflowers.

B. Oilseed Type. Black (some specialty varieties may be gray), thin-hulled, fairly large sized seed of the oil-producing variety of sunflower plant

Male-sterile lines

HA - maintainer

*any germplasm without fertility-restoring alleles.



RHA - restorer

*only a few stable sources of nuclear restoration.

Propagated by selfing, can make inbred lines

Propagated by selfing, can make inbred lines

K. Tsunewaki, 2003 – Crop Improvement: Alien Cytoplasms. *Encyclopedia of Applied Plant Sciences*

Male-sterile lines

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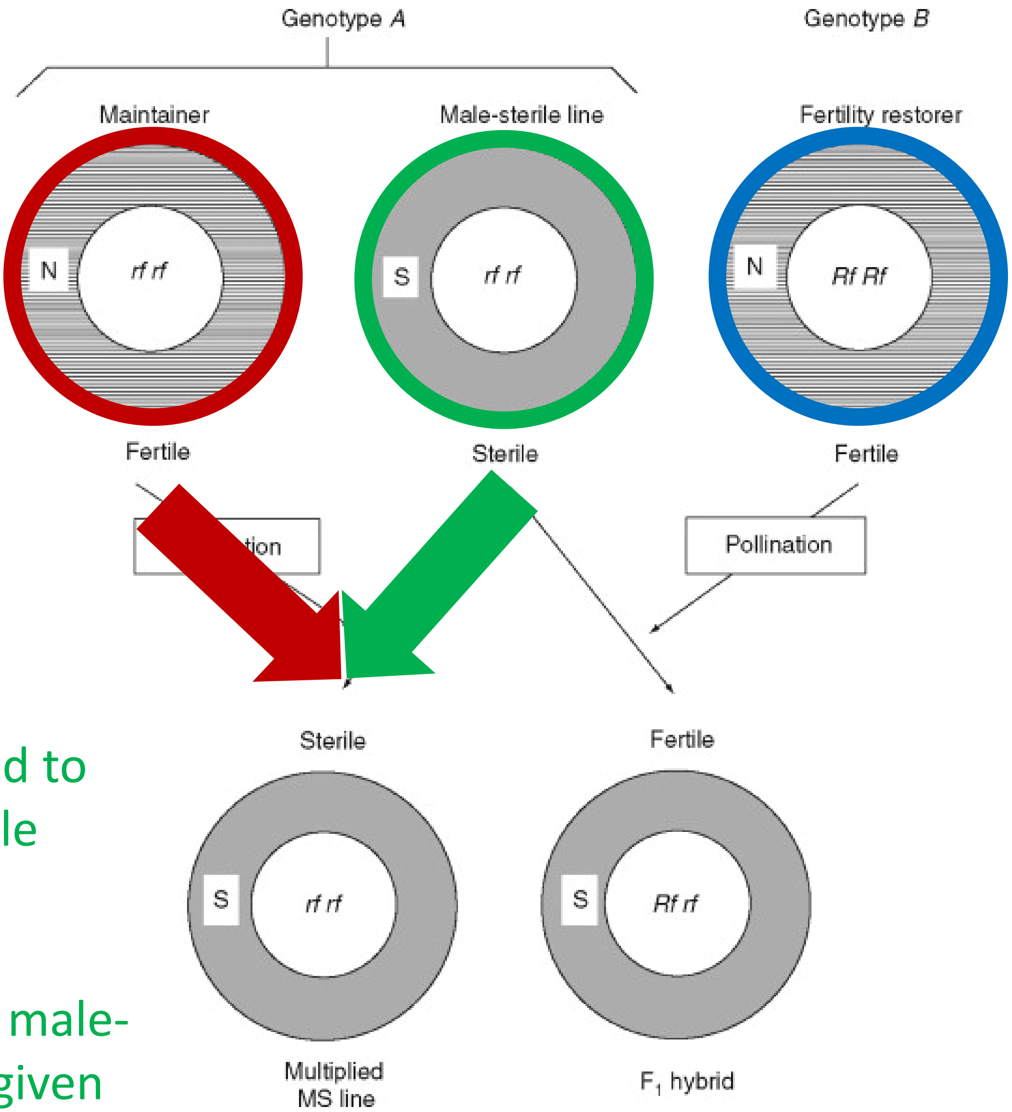


RHA - restorer

*only a few stable sources of nuclear restoration.

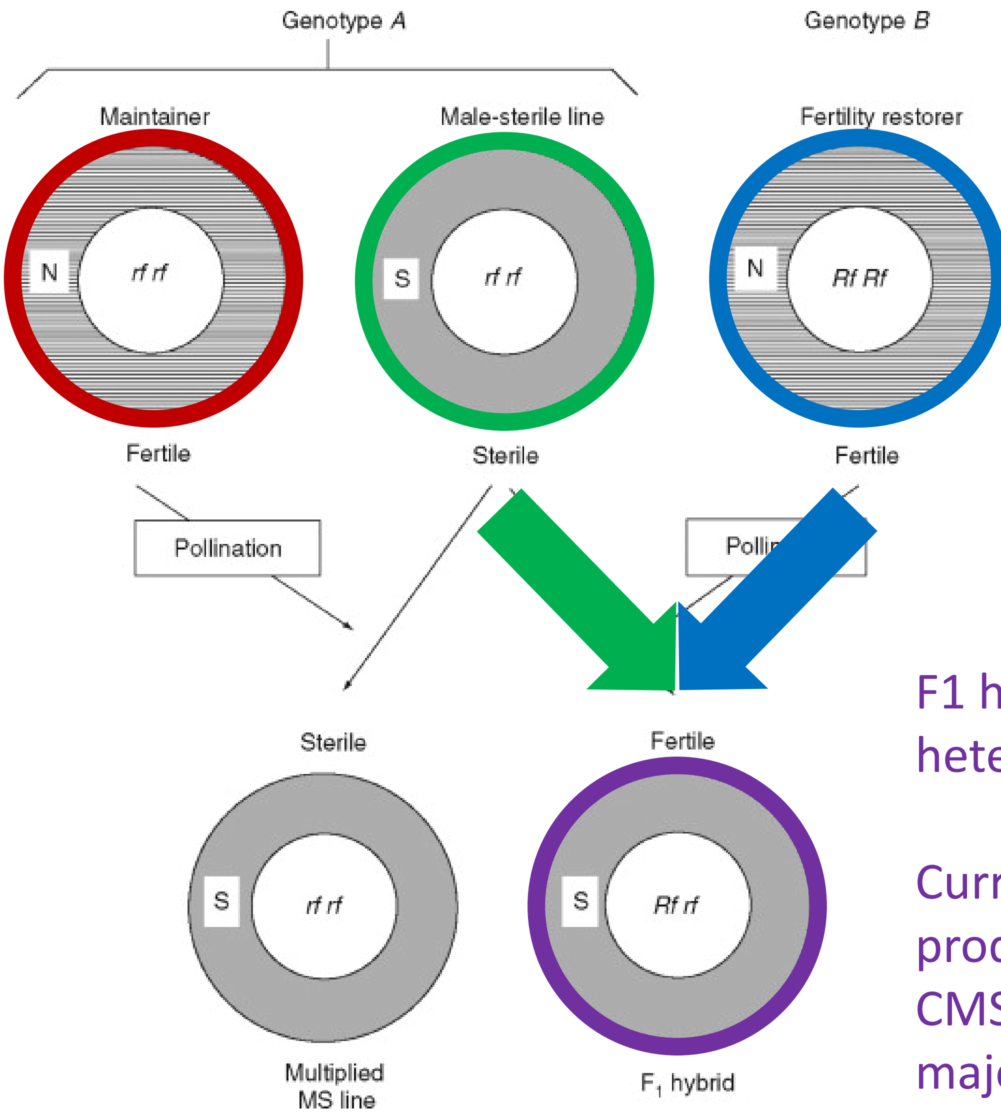
“Maintainers” are used to maintain the CMS-male sterile lines, and after several backcross generations you get a male-sterile version of any given “maintainer” genotype

K. Tsunewaki, 2003 – Crop Improvement: Alien Cytoplasms. *Encyclopedia of Applied Plant Sciences*

Male-sterile lines

HA - maintainer

*any germplasm without fertility-restoring alleles.



RHA - restorer

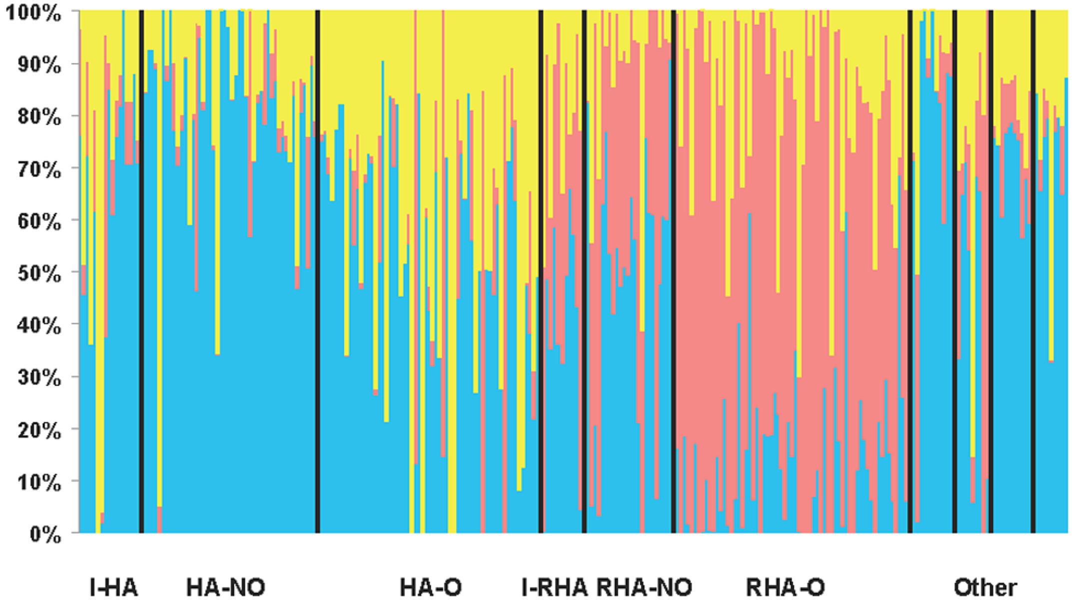
*only a few stable sources of nuclear restoration.

F1 hybrid seed with heterosis

Current commercial seed production mainly uses ONE CMS source (*PET1*) and ONE major fertility restoring gene – *Rf1*

K. Tsunewaki, 2003 – Crop Improvement: Alien Cytoplasms. *Encyclopedia of Applied Plant Sciences*

Genetic Divergence in Breeding Groups/Market Classes



PLOS GENETICS

OPEN ACCESS PEER-REVIEWED

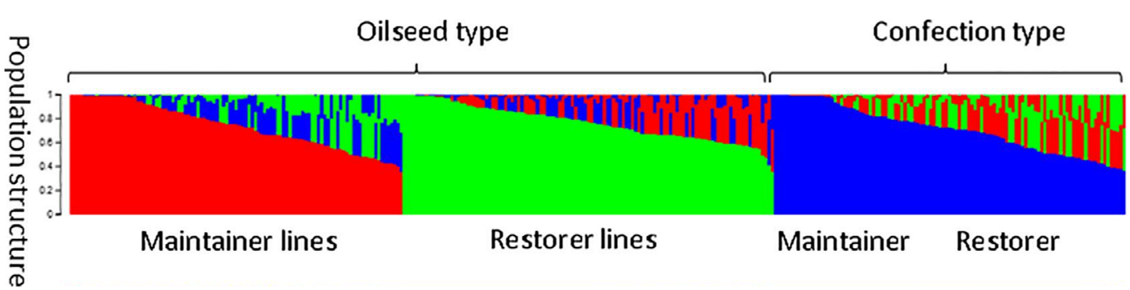
RESEARCH ARTICLE

2013

Association Mapping and the Genomic Consequences of Selection in Sunflower

Jennifer R. Mandel, Savithri Nambeesan, John E. Bowers, Laura F. Marek, Daniel Ebert, Loren H. Rieseberg, Steven J. Knapp, John M. Burke

Published: March 21, 2013 • https://doi.org/10.1371/journal.pgen.1003378



STRUCTURE – Bayesian clustering algorithm on 5000-9000 SNPs

frontiers in Genetics

ORIGINAL RESEARCH
published: 14 March 2019
doi: 10.3389/fgene.2019.00216

2019

Linkage Mapping and Genome-Wide Association Studies of the *Rf* Gene Cluster in Sunflower (*Helianthus annuus* L.) and Their Distribution in World Sunflower Collections

*Zahirul I. Talukder[1], Guojia Ma[1], Brent S. Hulke[2], Chao-Chien Jan[2] and Lili Qi[2]**

[1] Department of Plant Sciences, North Dakota State University, Fargo, ND, United States, [2] Edward T. Schafer Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, Fargo, ND, United States

Today's Main Question

Given the known genetic divergence among breeding groups and market classes, have there been (unintended) phenotypic divergences?

Particular focus on:

leaf ecophysiology
leaf secondary metabolism
plant relative growth rate

Sunflower Association Mapping (SAM) Panel

288 cultivated lines representing an estimated 90% of allelic diversity within the USDA and INRA germplasm repositories. Mean date of introduction = 1990 ± 7yr (SD)

Multiple genetic maps, most recently full-genome resequencing data available for 261 lines, resulting in ~1.4 million SNP map (~3.6 Gb genome)

Handy “Core 12” set of lines representing ~half of allelic diversity within USDA + INRA germplasm

Theor Appl Genet (2011) 123:693–704
DOI 10.1007/s00122-011-1619-3

ORIGINAL PAPER

Genetic diversity and population structure in cultivated sunflower and a comparison to its wild progenitor, *Helianthus annuus* L

J. R. Mandel · J. M. Dechaine · L. F. Marek · J. M. Burke

2011

Leaf Ecophysiology + Secondary Metabolism

288 lines grown under high-resource conditions

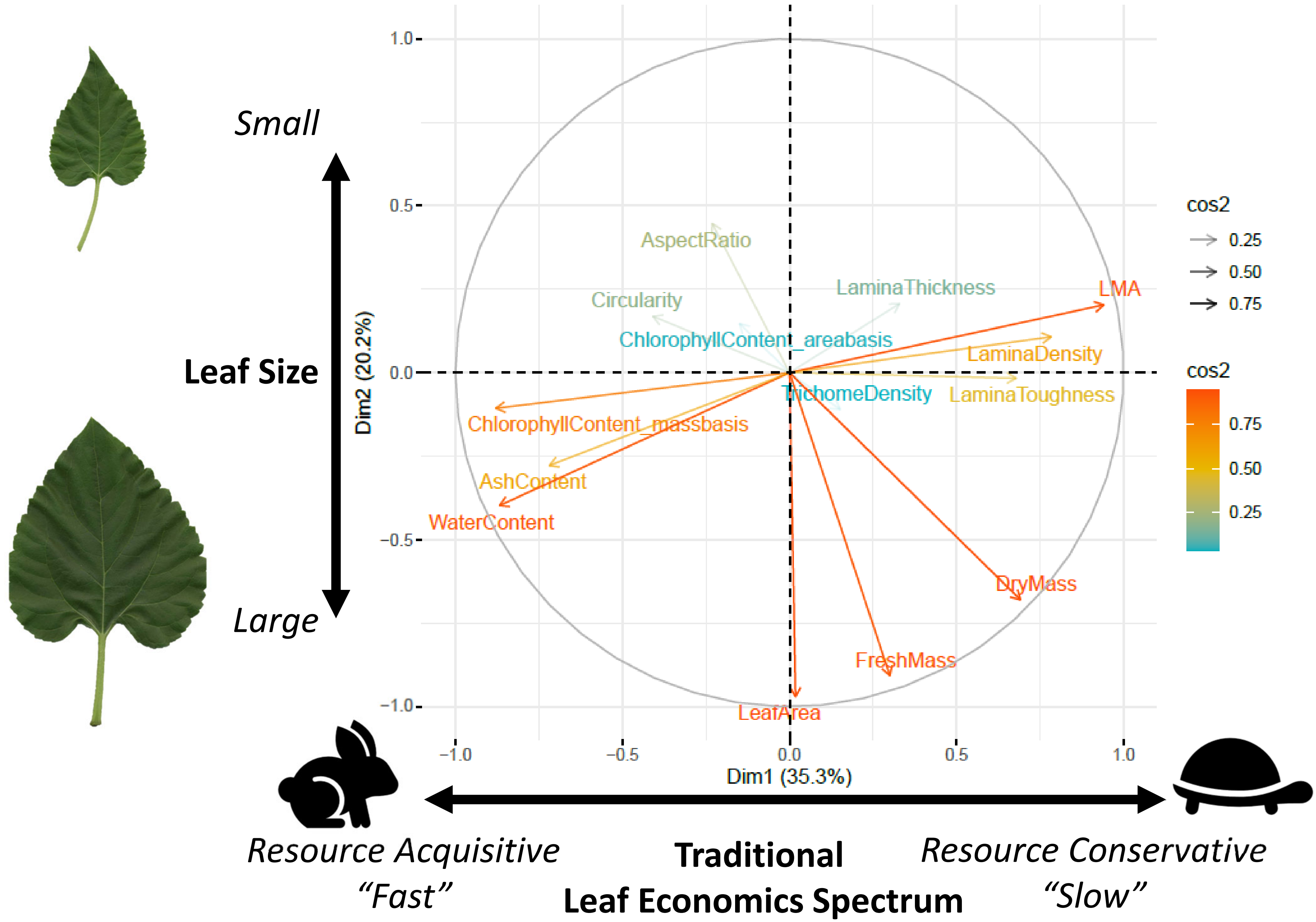
Pest/pathogen-free environment
(constitutive ecophysiology and metabolite production)

Sampling standardized by stage (V8-V10)

Pair of MRFELs taken for trait assessment
common ecophysiological traits
snap-frozen tissue analyzed by HPLC

Leaf Ecophysiology

PC1 – leaf economics (35%)
PC2 – leaf size (20%)

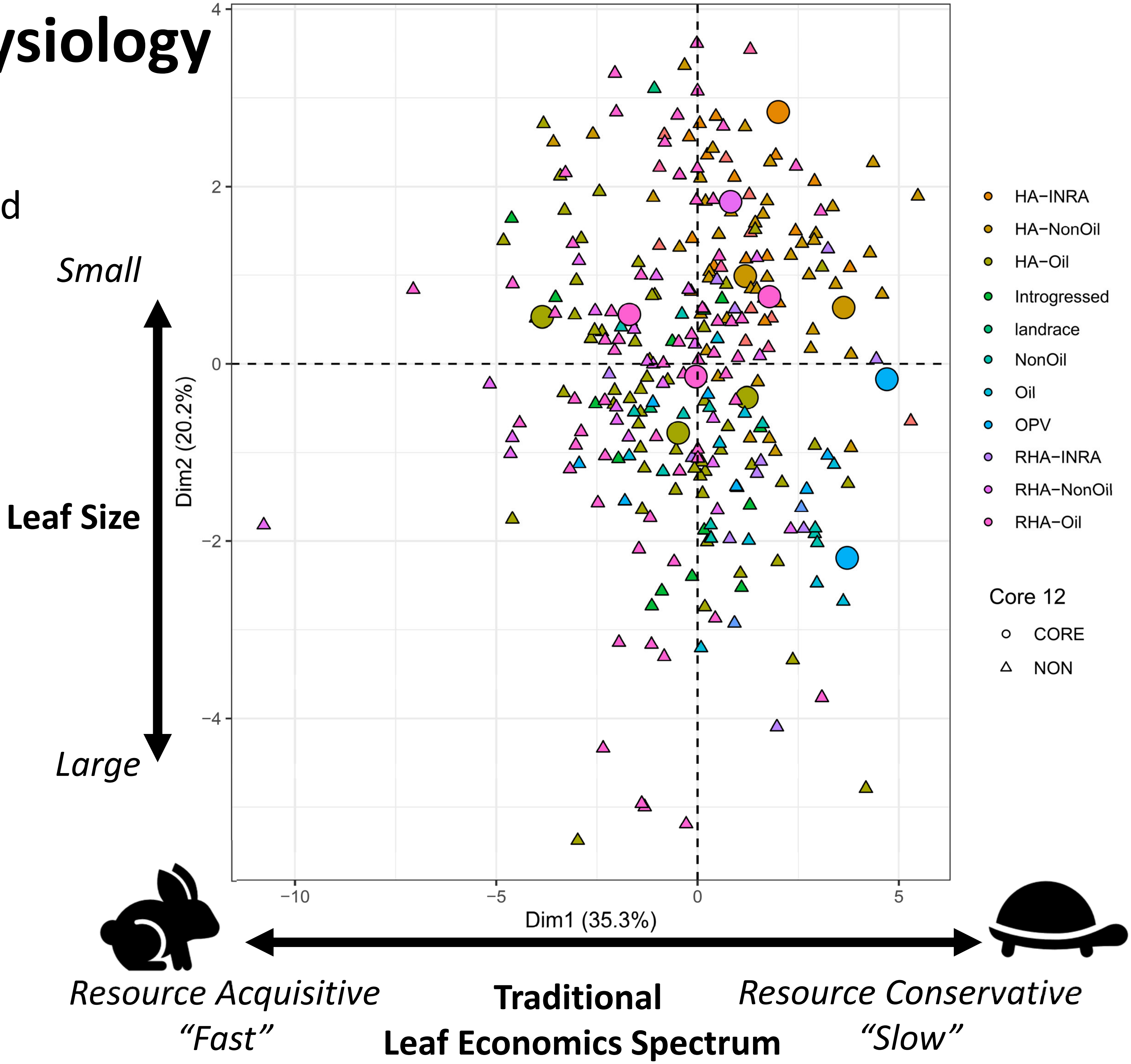


Leaf Ecophysiology

Interspersion of breeding groups and market classes, small differences

For **leaf economics (PC1)**, RHA on average “faster” than HA, effect size d=0.09 (Bayes Factor=51)

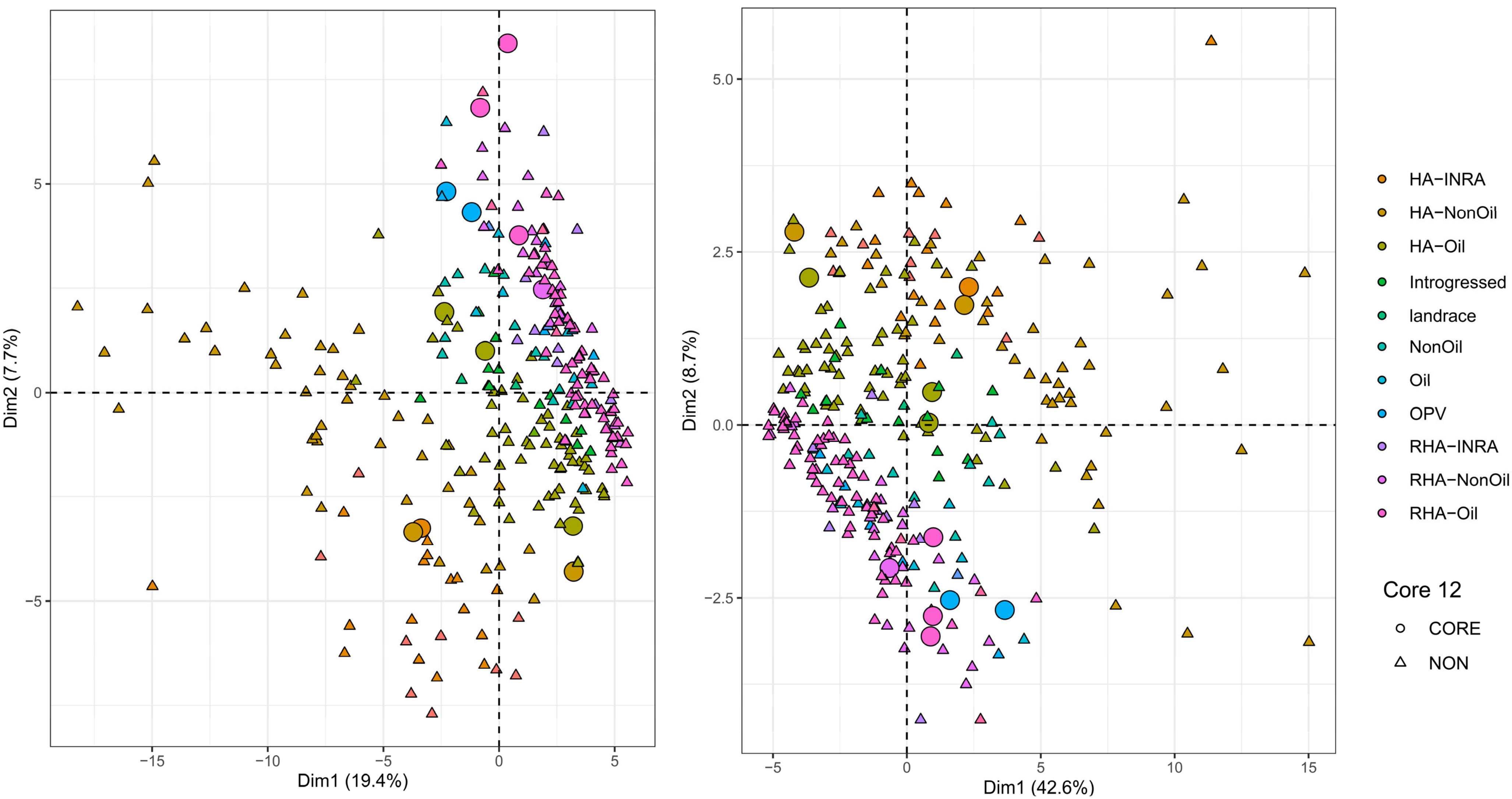
Oilseed lines had **larger leaves** than NonOil, effect size d=0.13 (BF=31)



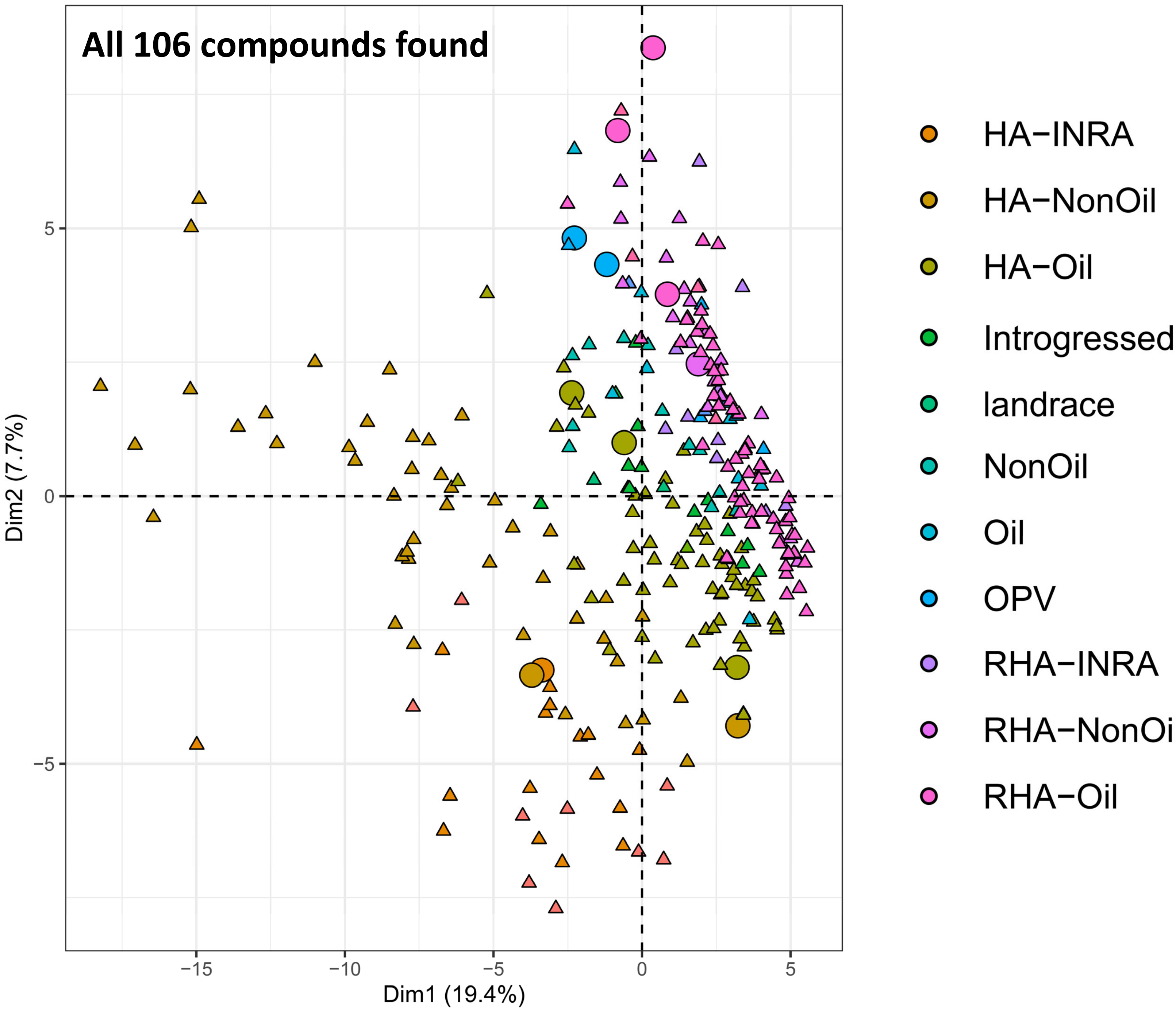
Leaf Secondary Metabolism (nonvolatile – HPLC)

All 106 compounds found
2 PCs = 27% of variation

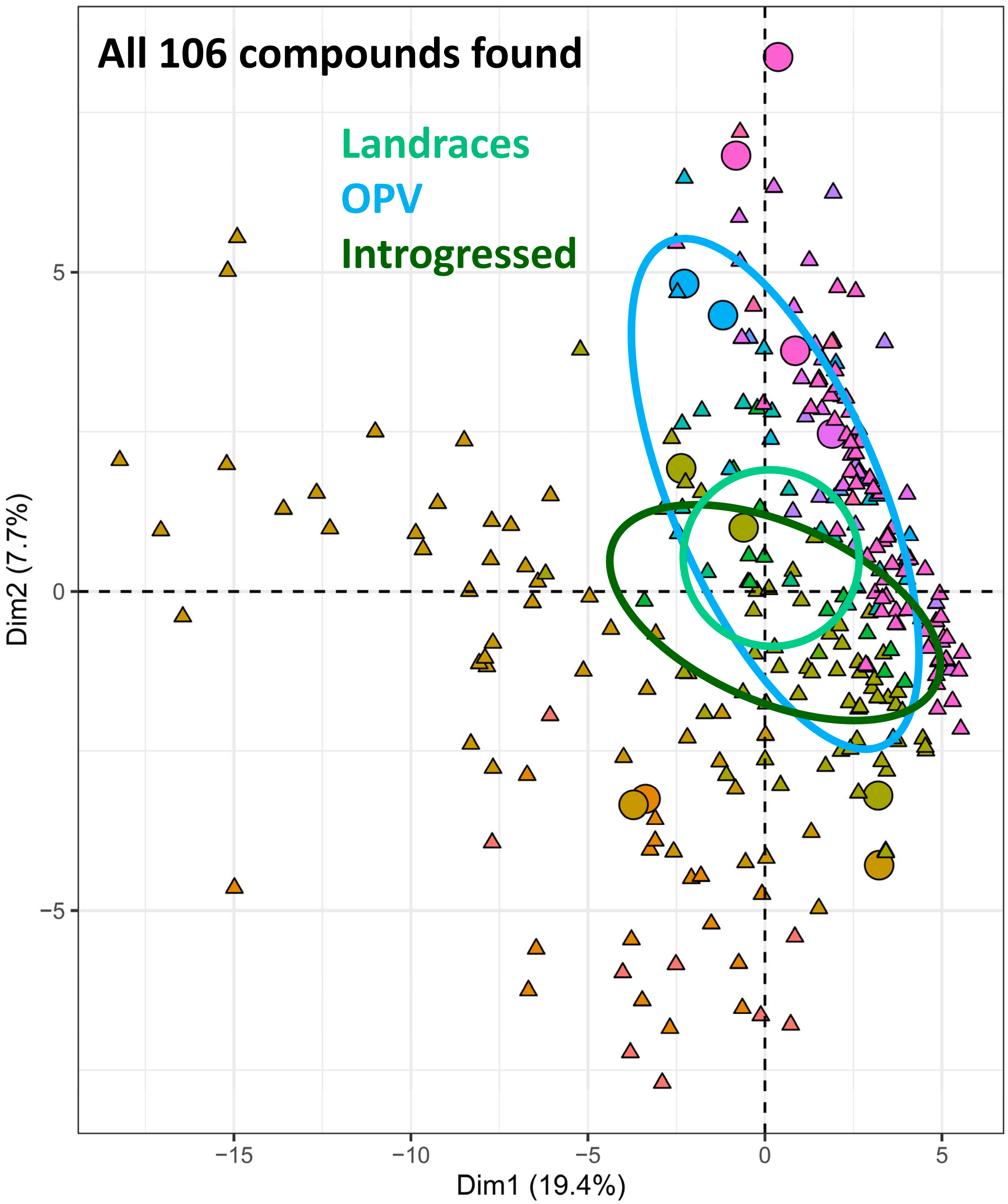
32 metabolites found
across all genotypes
2 PCs = 51% of variation



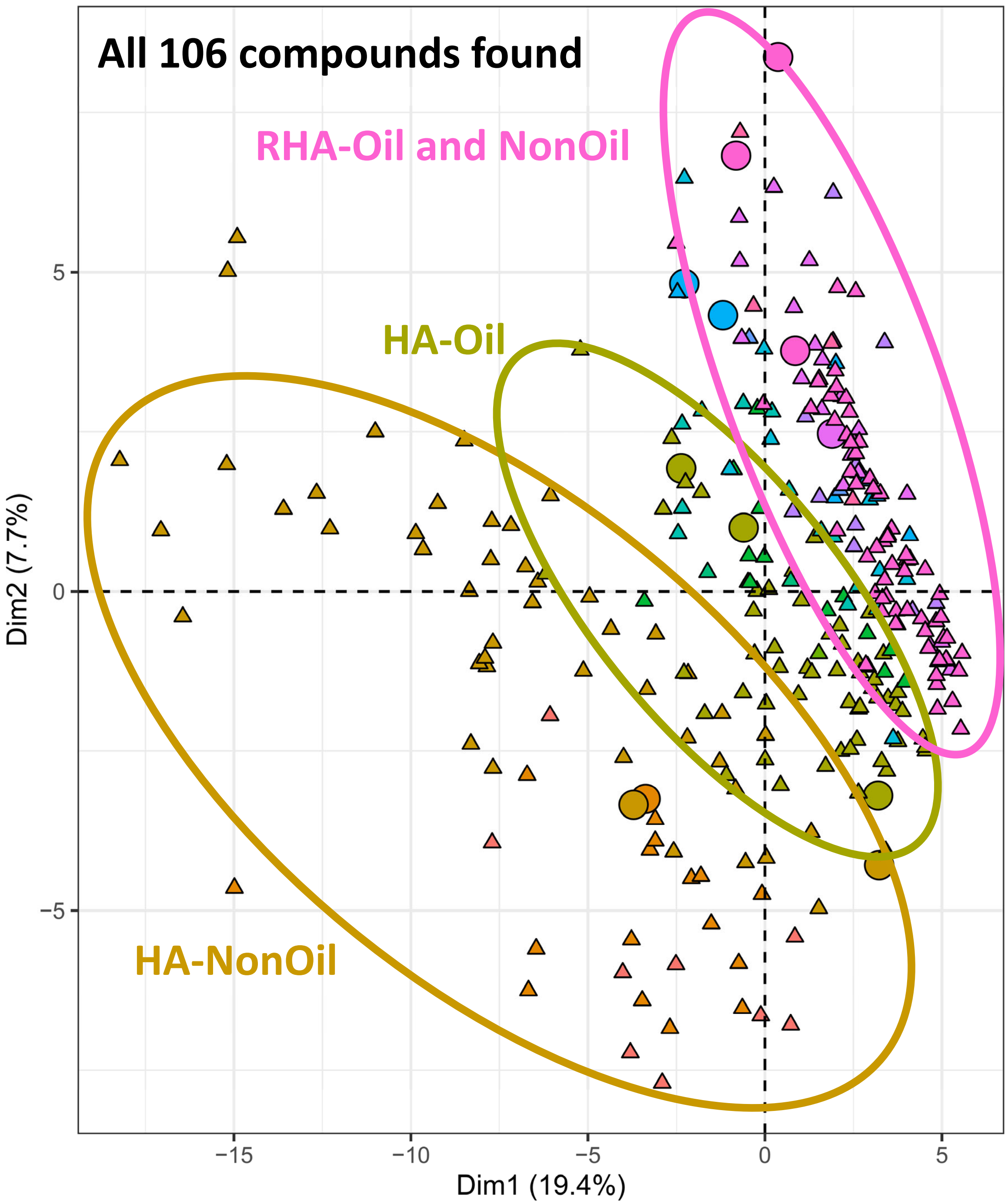
Leaf Secondary Metabolism (nonvolatile – HPLC)



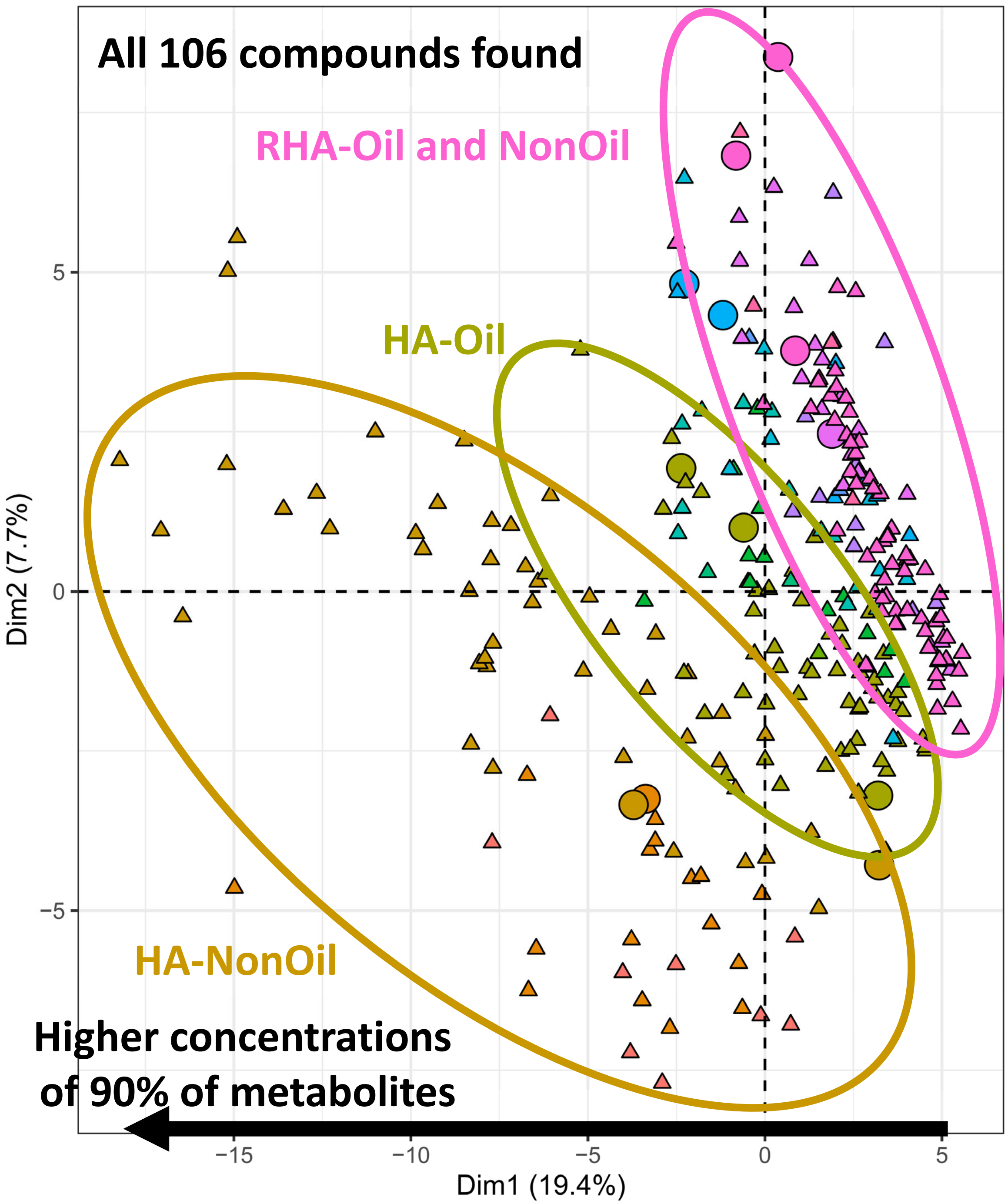
Leaf Secondary Metabolism (nonvolatile – HPLC)



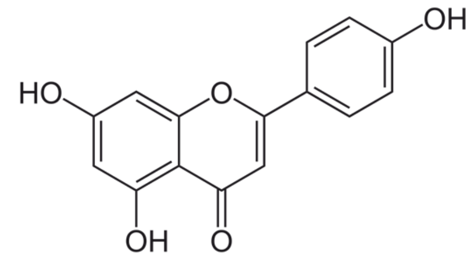
Leaf Secondary Metabolism (nonvolatile – HPLC)



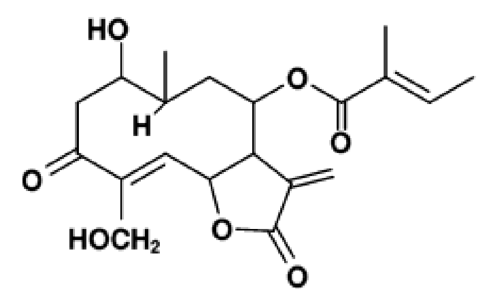
Leaf Secondary Metabolism (nonvolatile – HPLC)



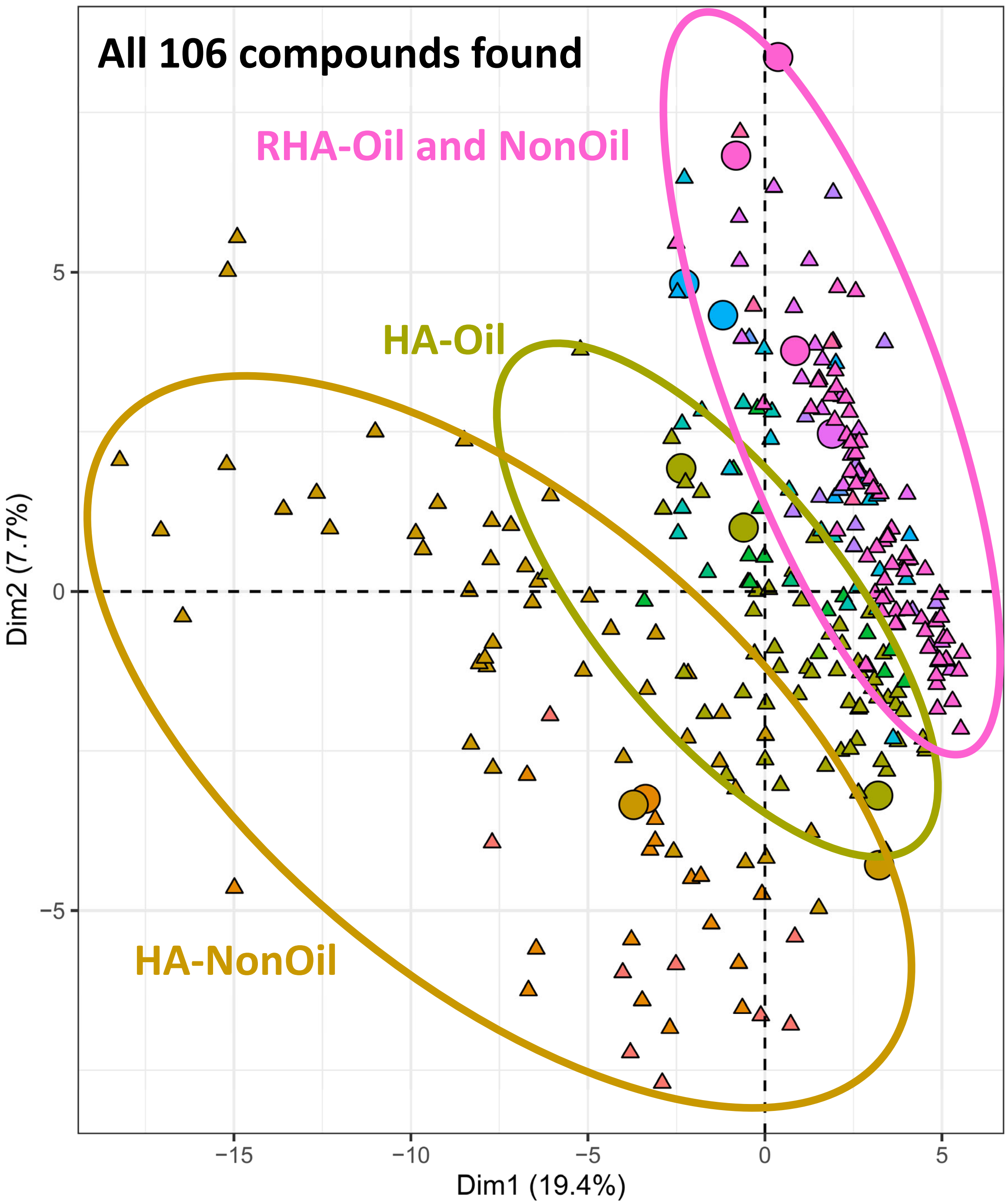
Higher relative concentrations of flavonoids



Higher relative concentrations of sesquiterpene lactones



Leaf Secondary Metabolism (nonvolatile – HPLC)



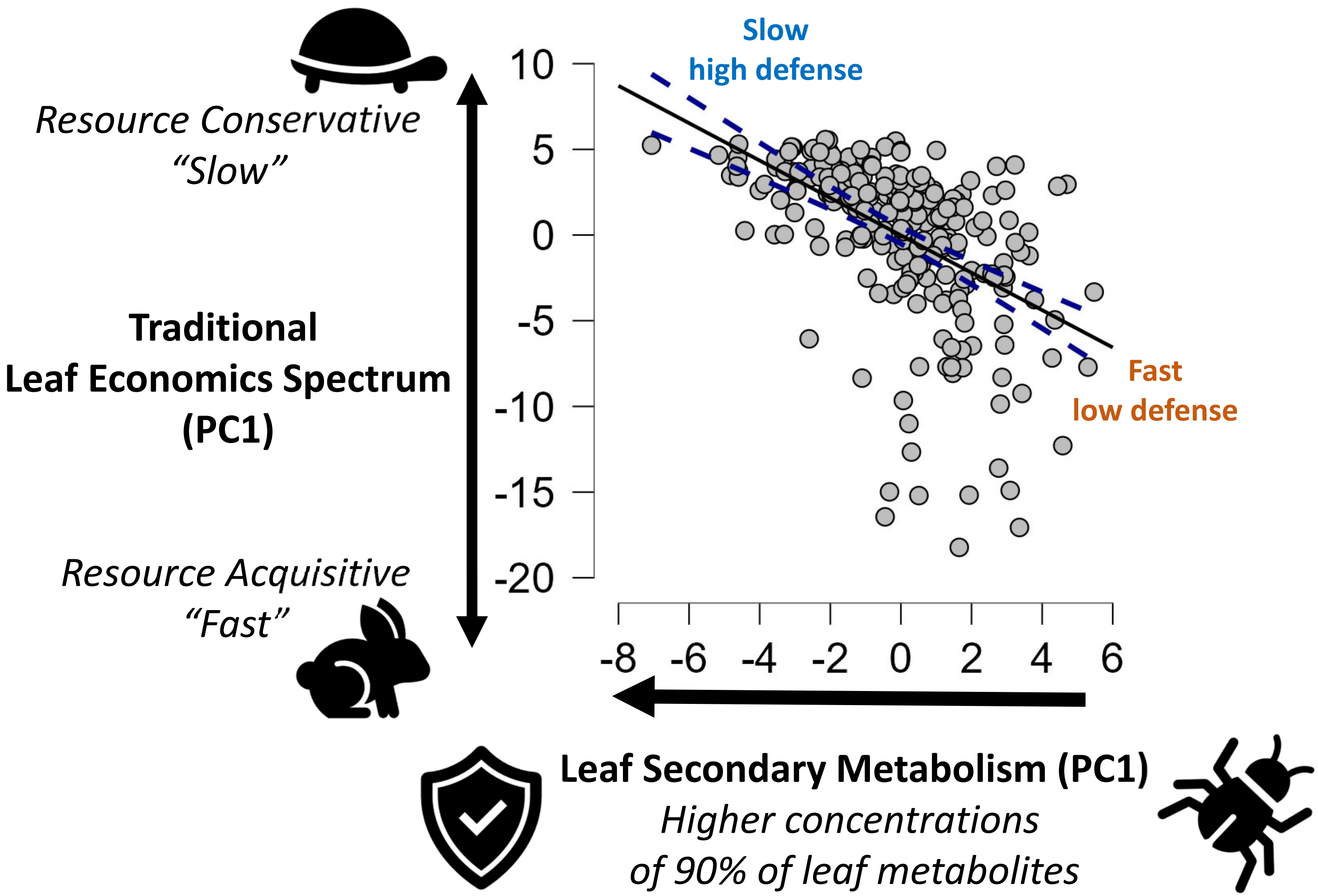
HA-maintainer lines have on average higher concentrations of nonvolatile secondary metabolites than RHA lines – effect size d=0.24 (BF>1000)

NonOil varieties have on average higher concentrations of nonvolatile secondary metabolites than Oilseed lines – effect size d=0.23 (BF>1000)

RHA-restorer lines have on average higher flavonoids and HA lines have higher SQTLs – effect size d=0.21 (BF>1000)

Leaf Ecophysiology + Secondary Metabolism

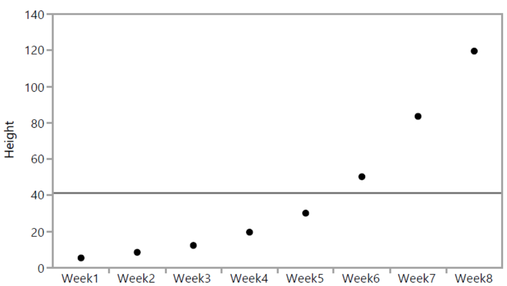
Negative correlation between <u>leaf economic strategy</u> and overall secondary metabolite <u>concentrations</u> (R^2=0.25) among lines

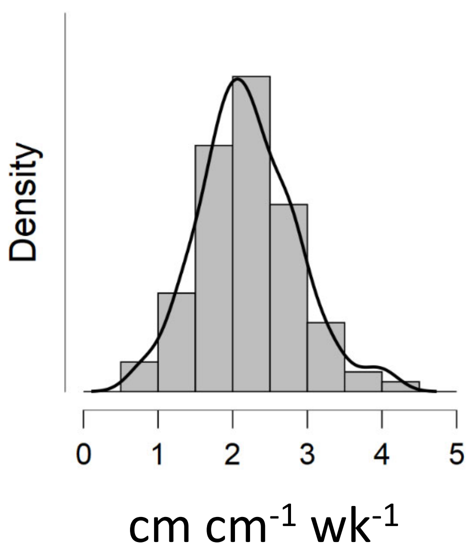


Height-based relative growth rate (RGR)

SAM panel grown in a randomized complete block design across two high tunnels under uniform high-resource conditions.

Height assessed weekly for 8 weeks. From this, relative growth rate during peak growth (weeks 3-6) was calculated.





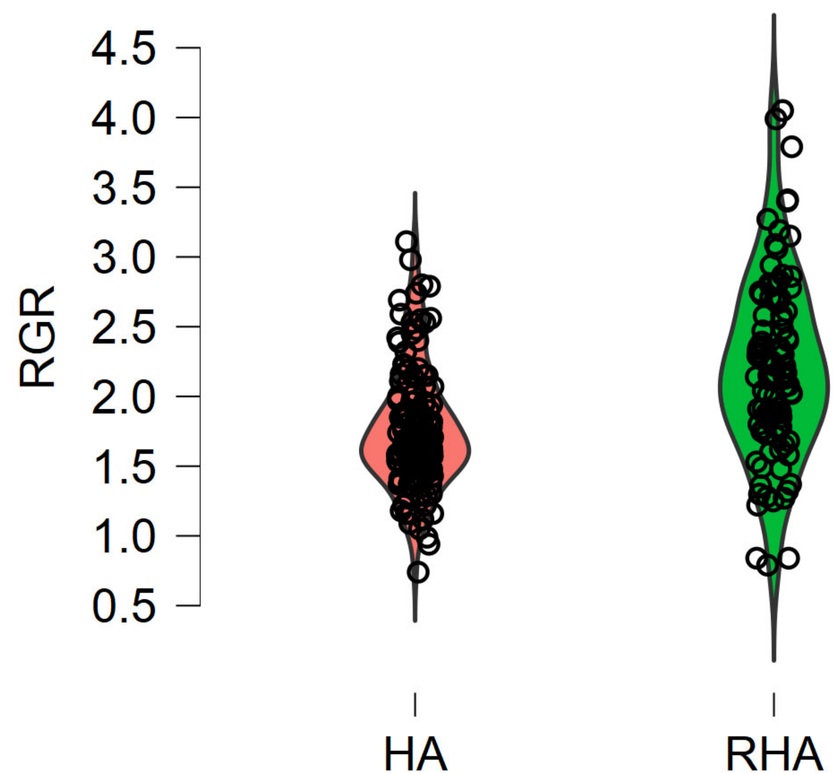
We found a large difference in average Relative Growth Rate between **RHA-restorer** and **HA-maintainer** breeding groups, with RHA having ~30% faster average inherent growth rate than HA.

<u>RHA>HA, effect size d=0.79 (BF>1000)</u>

RHA 2.2 cm cm^{-1} wk^{-1} [2.1-2.3] 95% Cr.I.

HA 1.7 cm cm^{-1} wk^{-1} [1.7-1.8] 95% Cr.I.

No support for overall differences in RGR between Oil and NonOil lines.



So overall…

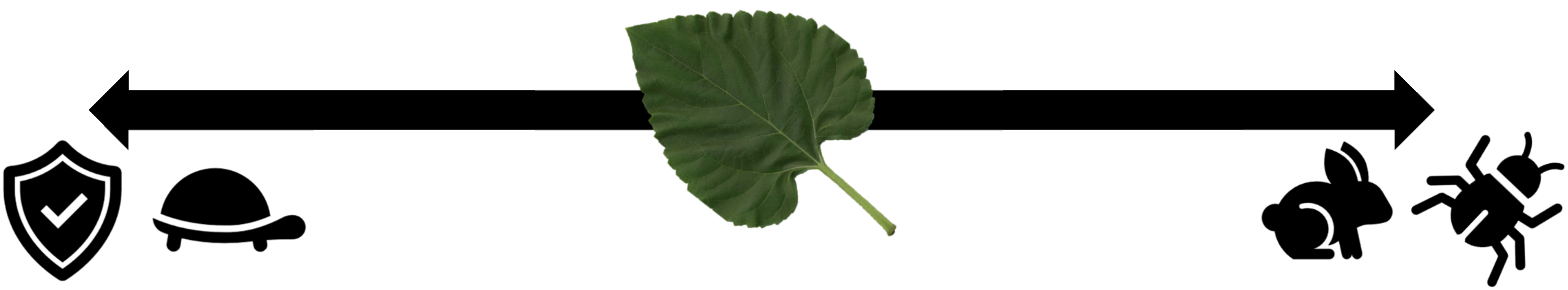
HA-maintainer and **RHA-restorer** lines have diverged in leaf ecophysiology, plant relative growth rate, and *especially* constitutive nonvolatile leaf secondary metabolism.

HA – maintainer

more resource-conservative leaves
lower RGR
higher conc. of metabolites
more SQTLs relative to flavonoids

RHA – restorer

more resource-acquisitive leaves
higher RGR
lower conc. of metabolites
more flavonoids relative to SQTLs



But *WHY?* Two potential hypotheses:

(1) **HA-maintainer** lines are more heavily used in breeding efforts for specific target traits, given the ease of performing experimental crosses using male-sterile lines. This, coupled with limited mixing between RHA and HA breeding groups, may explain groups diverging over time and HA diverging farther from OPVs/landraces.

(2) Shifts in breeding goals over time from yield maximization to yield regularity? **RHA-restorer** 'faster' leaf ecophysiology, faster growth, and lower metabolite content may reflect older goals of maximizing total yield, while **HA-maintainer** 'slower' leaf ecophysiology, slower growth, and higher metabolite content may reflect more recent goals of improved yield regularity.

Strong parallels to sunflower wild relatives!

Across wild *Helianthus*, we see very similar axes of trait divergence.

Resource conservative leaves = lower RGR = higher constitutive chemical defense. This suggests that similar constraints on plant form and function exist under artificial selection in crop sunflower.

Next steps – inducible secondary metabolism!

This work focused on <u>constitutive</u> secondary metabolism. Moving forward, we are using the SAM panel to map the genetic architecture underlying inducible secondary metabolism under attack by a range of pests and pathogens. We hope to determine:

(1) what inducible chemical defenses are 'universal' in crop sunflower, versus divergent within the cultivated germplasm?

(2) what inducible chemical defenses are enemy-specific, versus generally activated under attack by multiple pests/pathogens?

(3) are there novel inducible chemical defenses in wild *Helianthus* that could help contribute to polyresistance against enemies like chewing insects and necrotrophic pathogens?

FOUNDATION FOR FOOD & AGRICULTURE RESEARCH

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Laura Marek @ USDA

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Questions?