Berry class synchronization: a prospective to understand berry ripening ?

Laurent Deluc
Horticulture Department (OSU) - August 25th 2011

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Two questions:

Do we have uniform clusters at harvest ?

How does it work?

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If not it may contribute to the wine balance

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Two questions:

Do we have uniform clusters at harvest ?

If not it may contribute to the wine balance

How does it work?

it could be useful to improve fruit quality

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Consequences of ripeness heterogeneity at harvest

- **Wine Quality :**

 - *presence of a non-negligible proportion of unripe berries can increase the appearance of bitter and astringent characters in wine and undesirable flavors*

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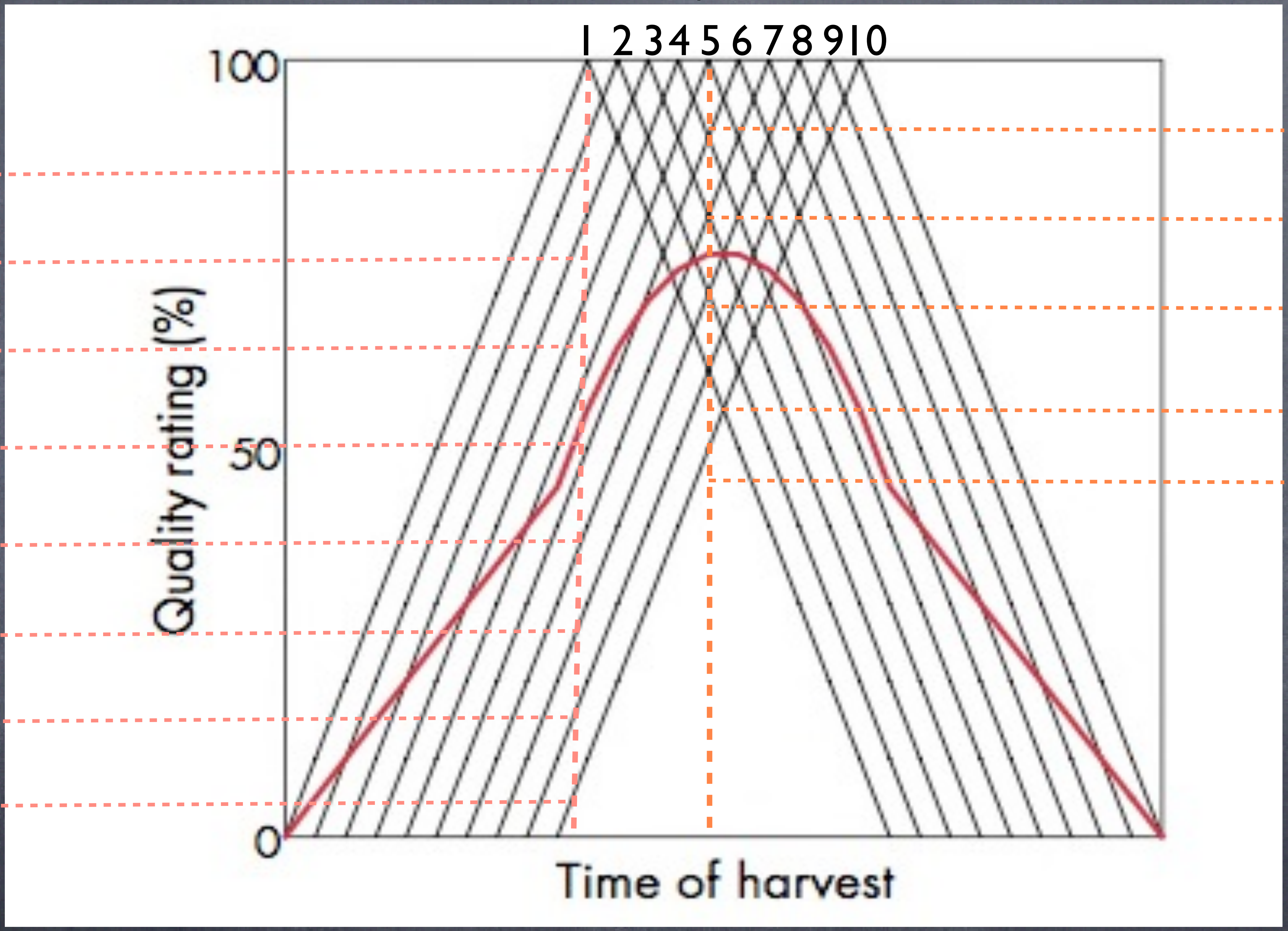
Contributions of different berry classes to the overall quality

Harvest date based on ripening rate of berry class # 1

1: 100%
2: 90%
3: 80%
4: 67%
5: 55%
6: 43%
7: 35%
8: 20%
9: 10%
10: 0%

Overall Quality = 50%



Gray 2006

Harvest date based on ripening rate of berry class #1

5: 100%
4,6: 92%
3,7: 82%
2,8: 68%
1,9: 55%
10: 50%

Overall Quality = 74%

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Consequences of ripeness heterogeneity at harvest

- ***Wine Balance :***

- presence of a non-negligible proportion of unripe berries can increase the appearance of bitter and astringent characters in wine and undesirable flavors

- ***Heterogeneity in phenolic maturity observed between different berry classes at harvest in Cabernet Sauvignon (Kontoudakis et al., 2011):***

- Higher sugar content is correlated to higher anthocyanin, total phenolic index, proanthocyanidin concentrations

- ***Few data are available...***

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Ripeness heterogeneity among berries may depend upon the genetic background

"Pinot Noir Berries"

"Zinfandel, Cabernet Sauvignon, Shiraz"

"Merlot Berries"

Low — Moderate — Extreme

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Is Pinot Noir able to alleviate berry variability at harvest?

?

Are under ripe berries at mid-véraison able to catch up more advanced berries and how?

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Reduction of berry class variability

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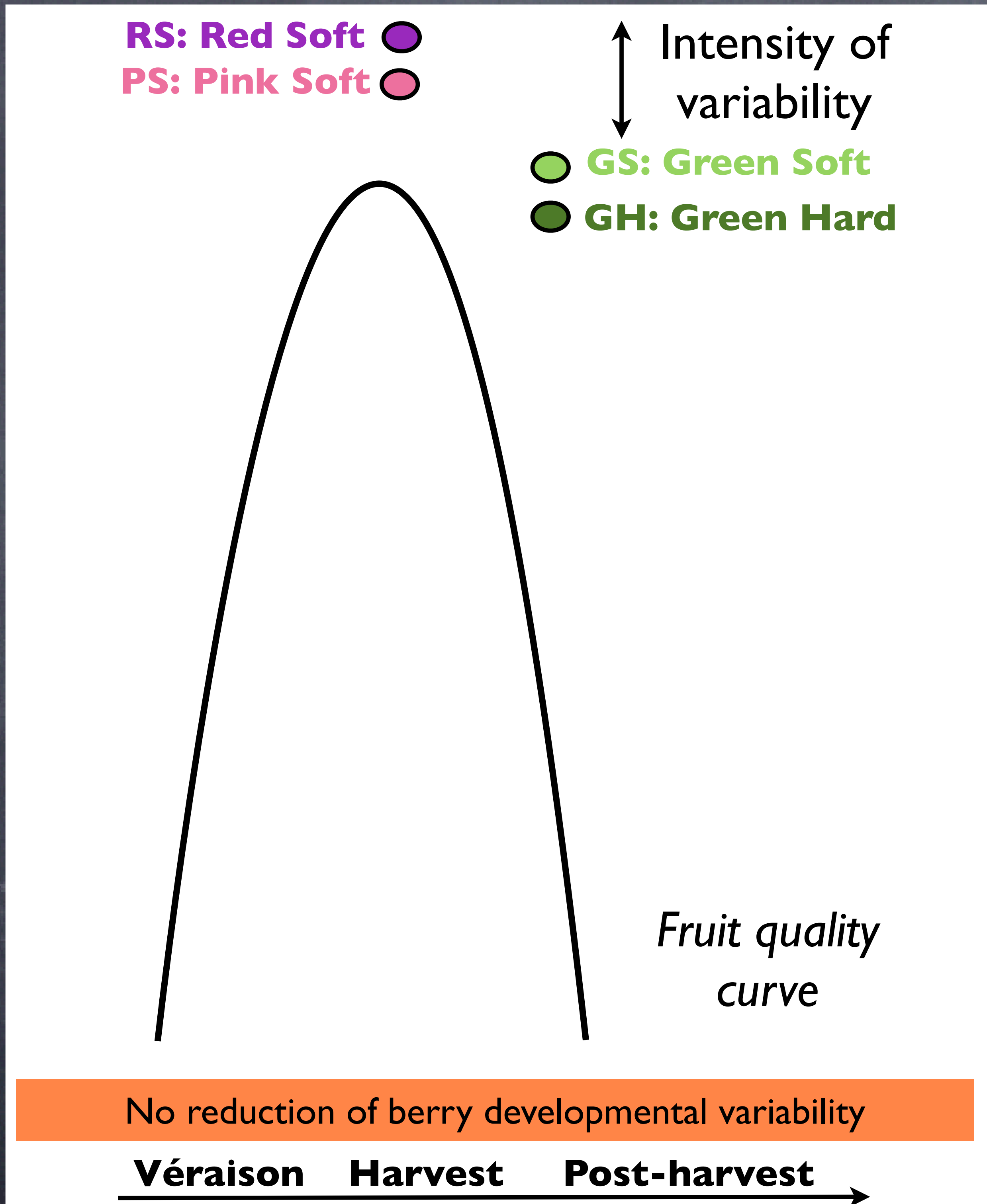

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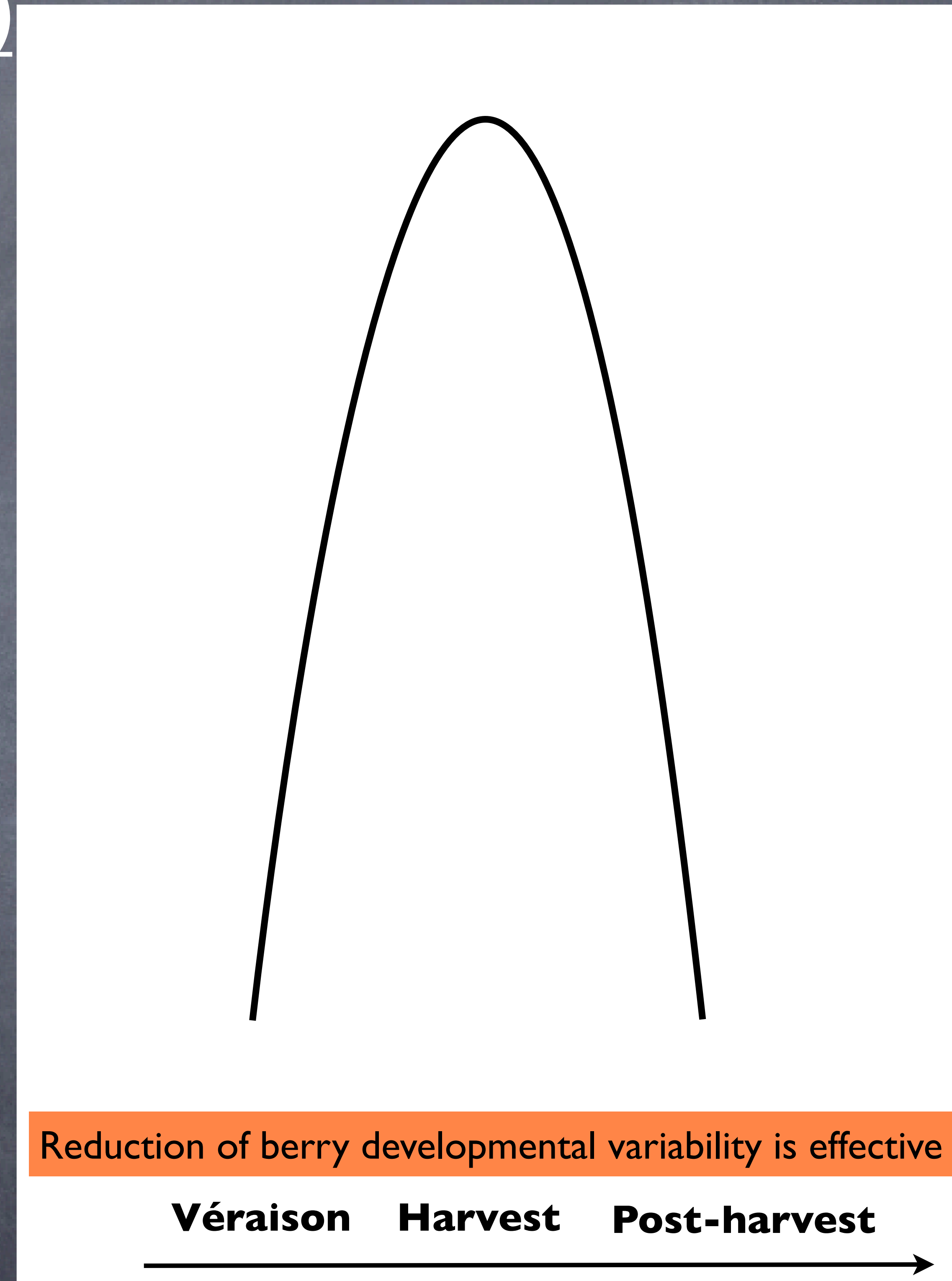

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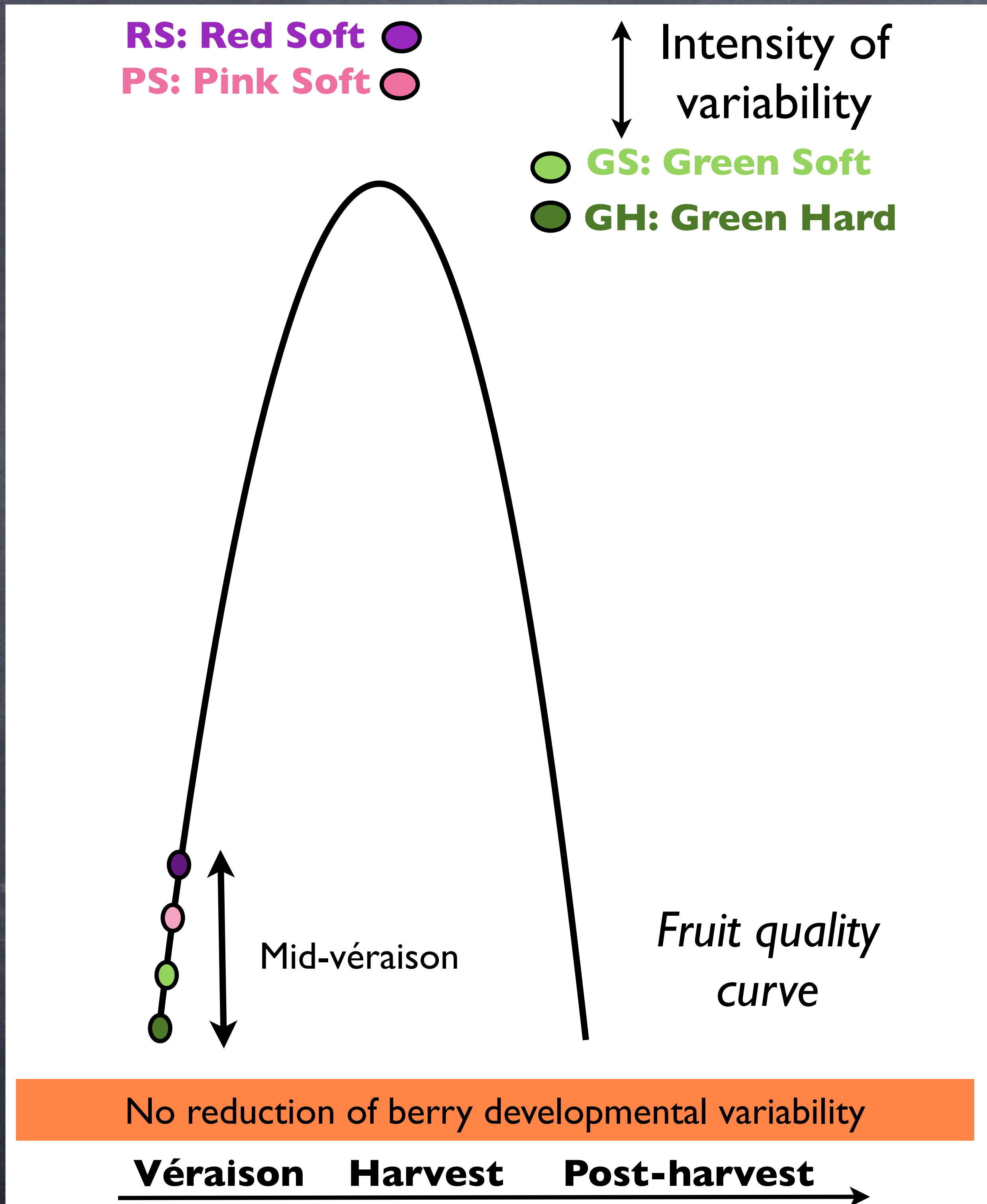

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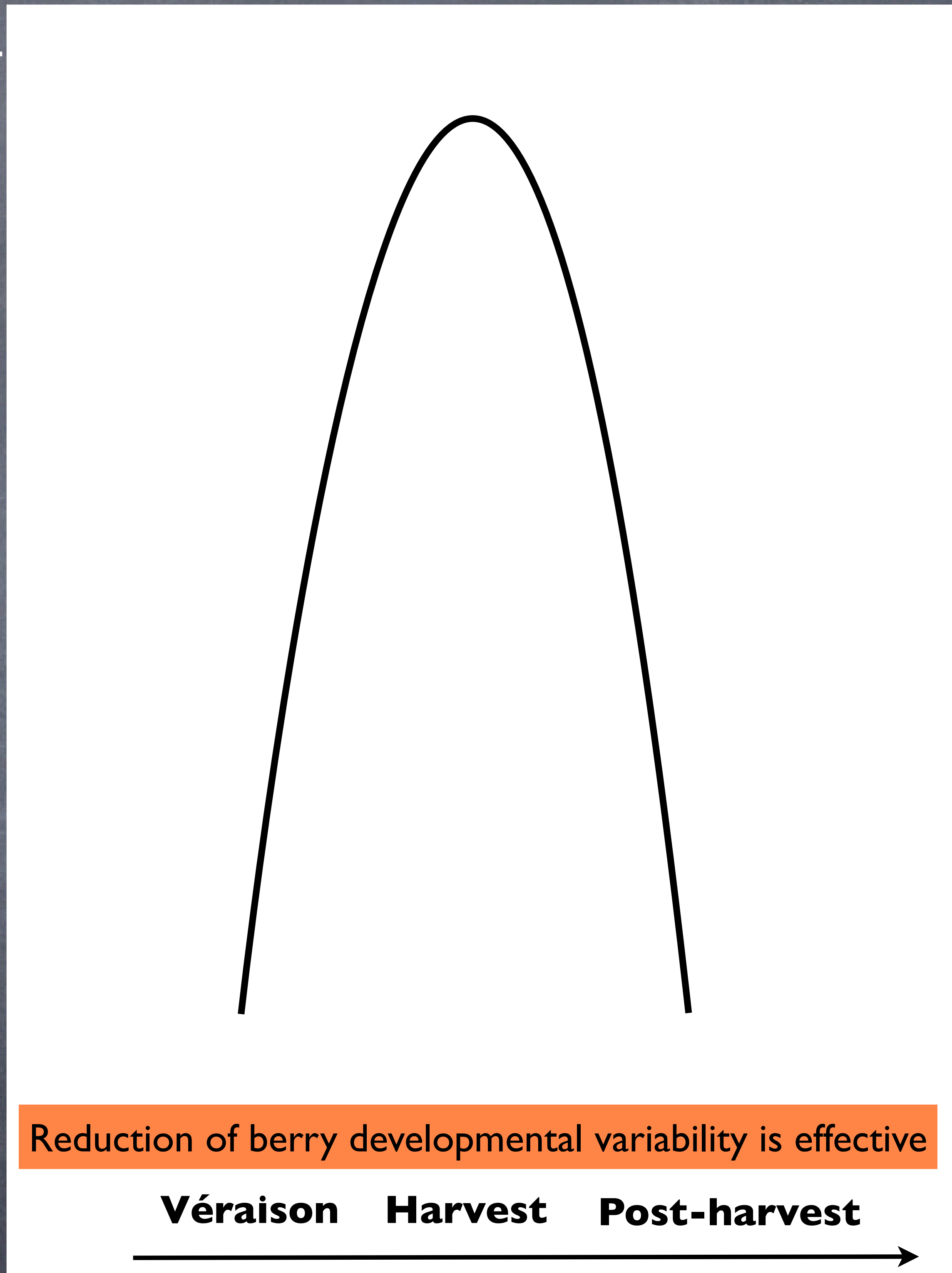

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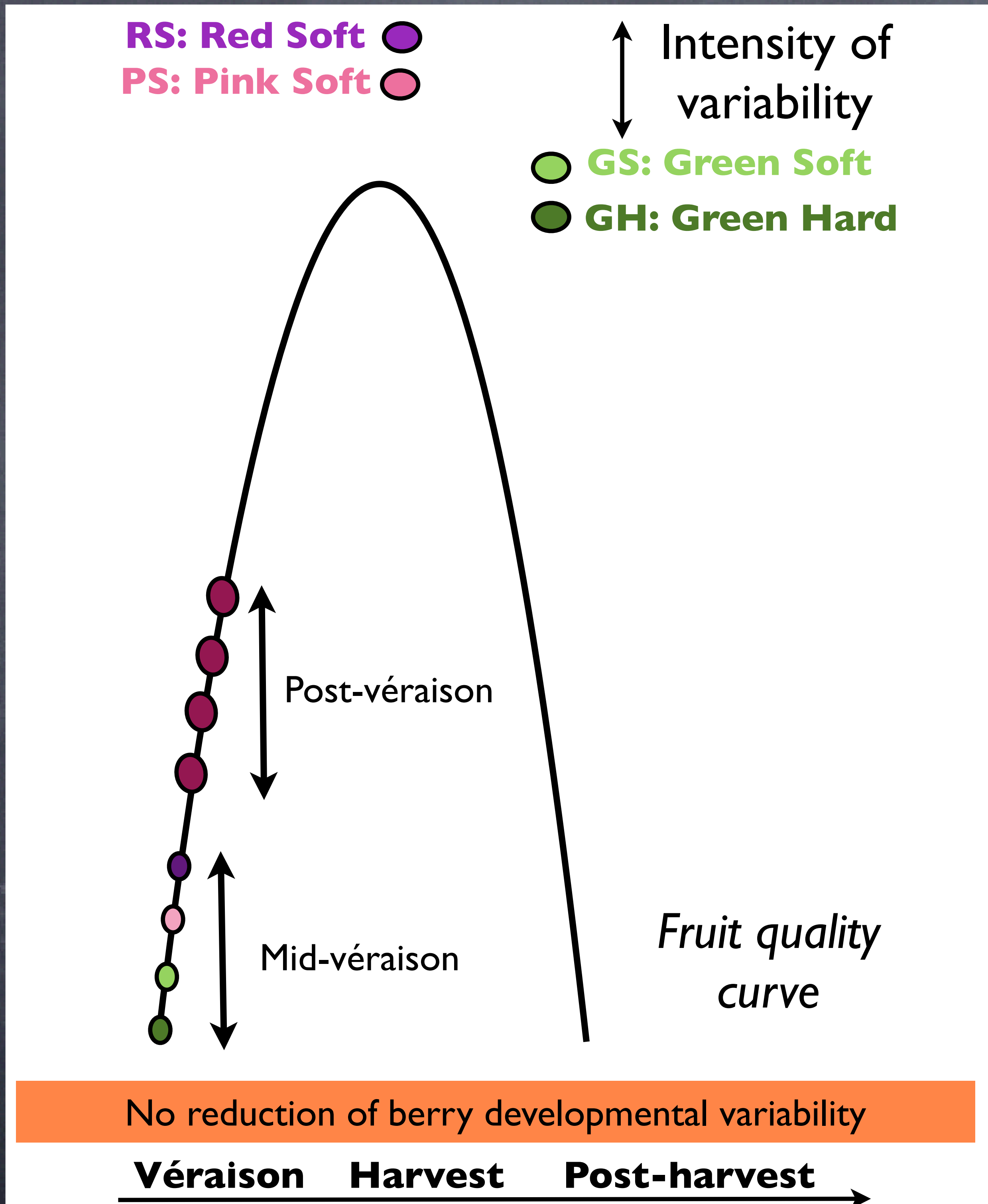

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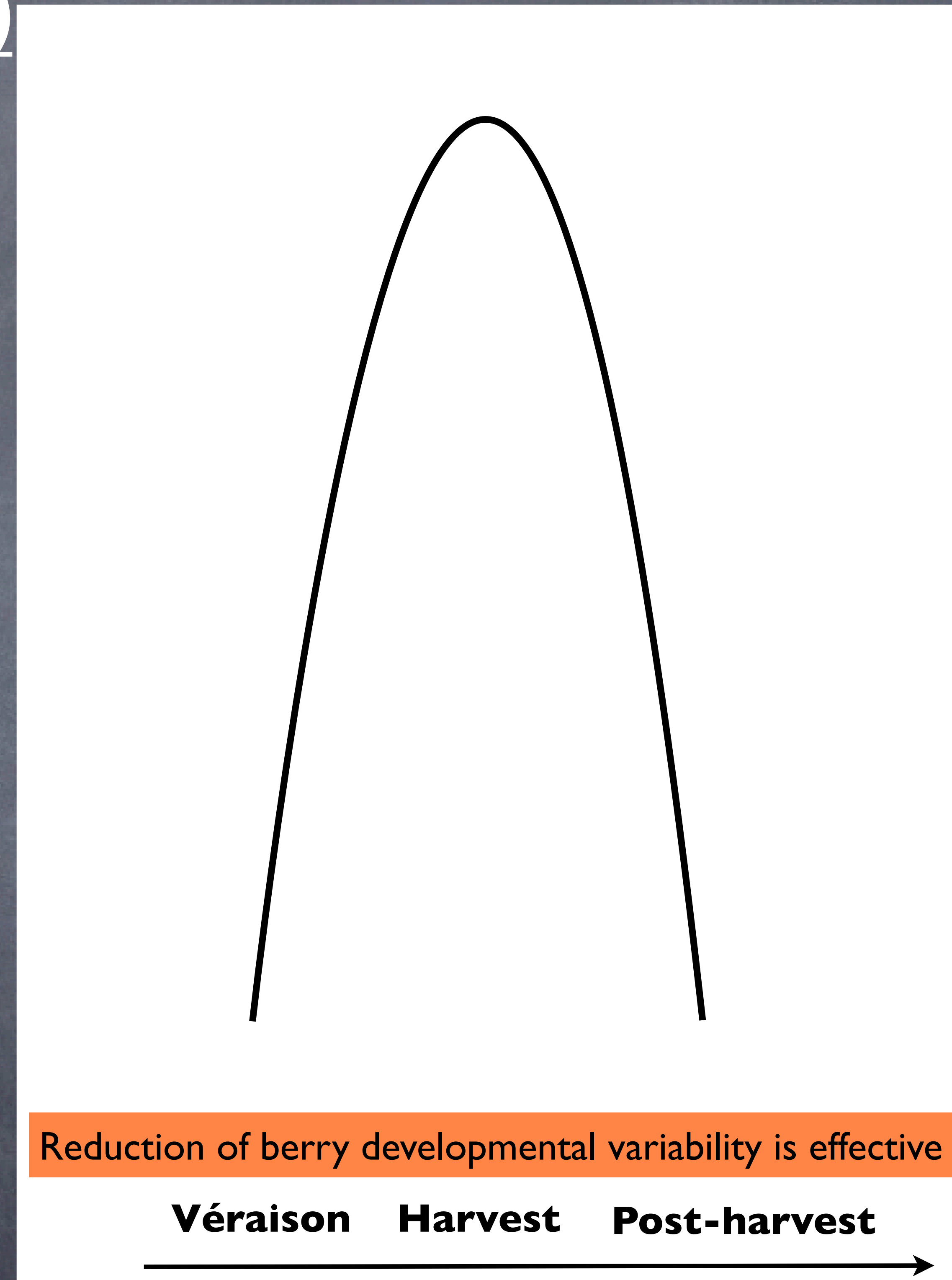

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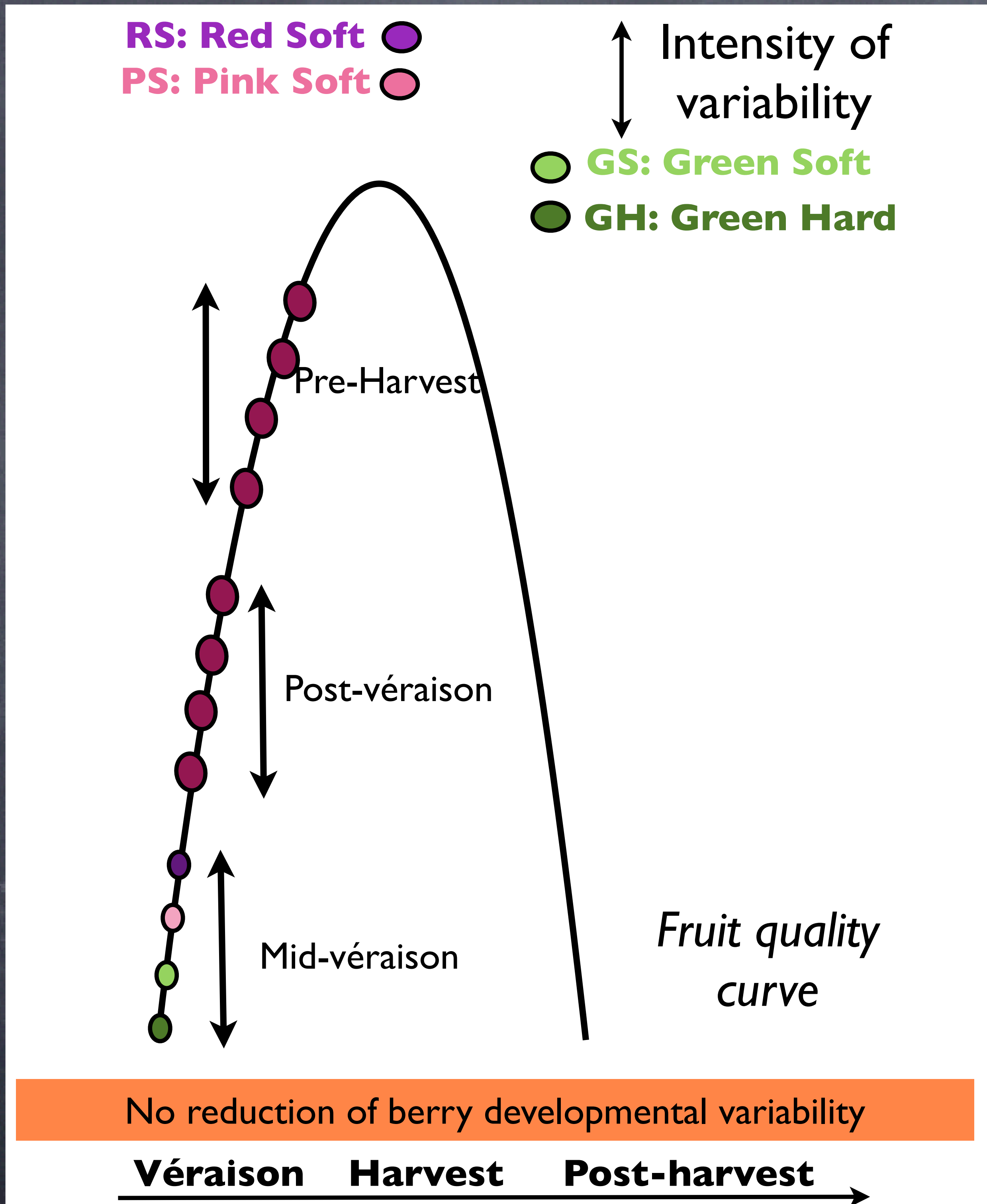

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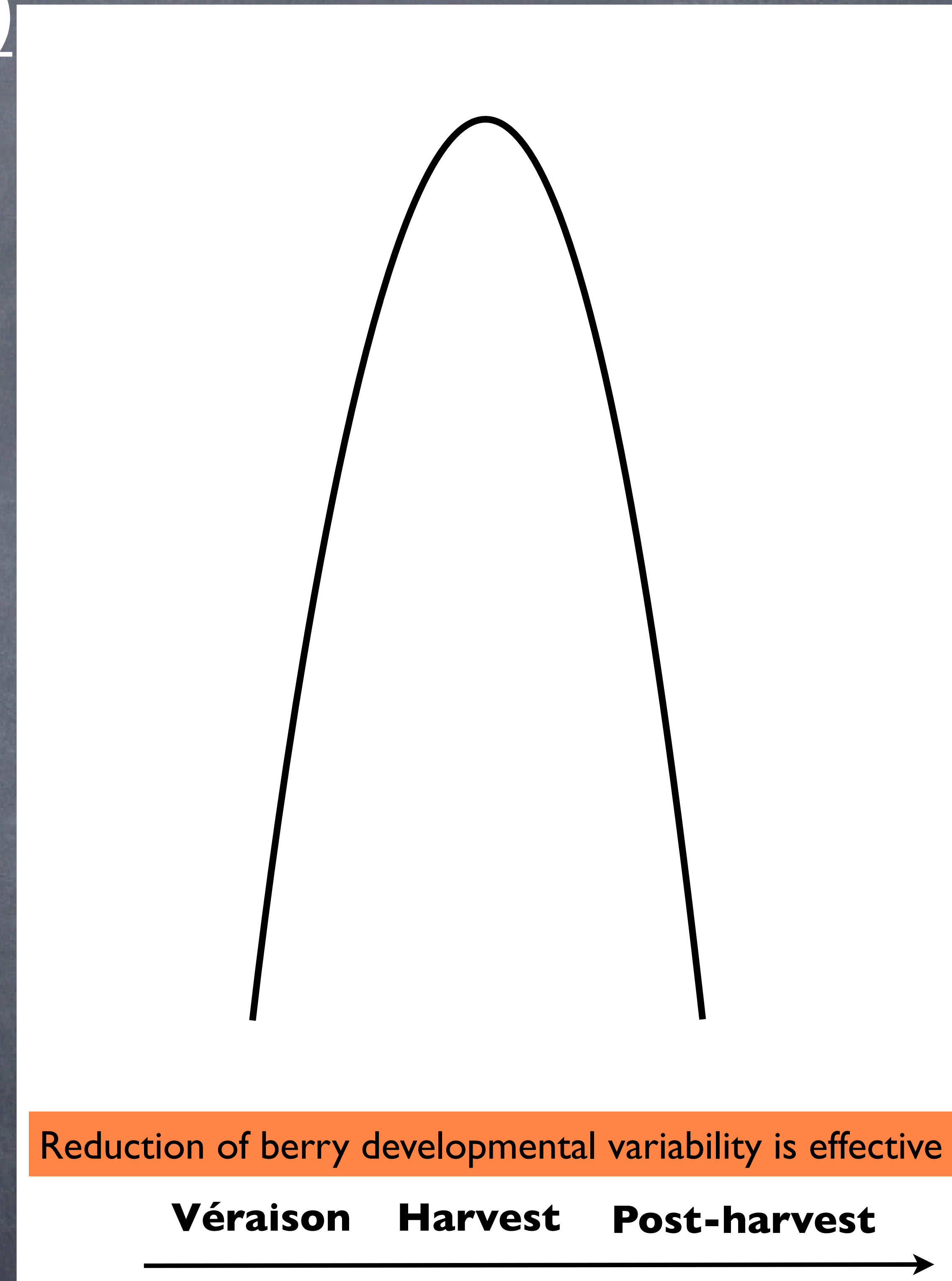

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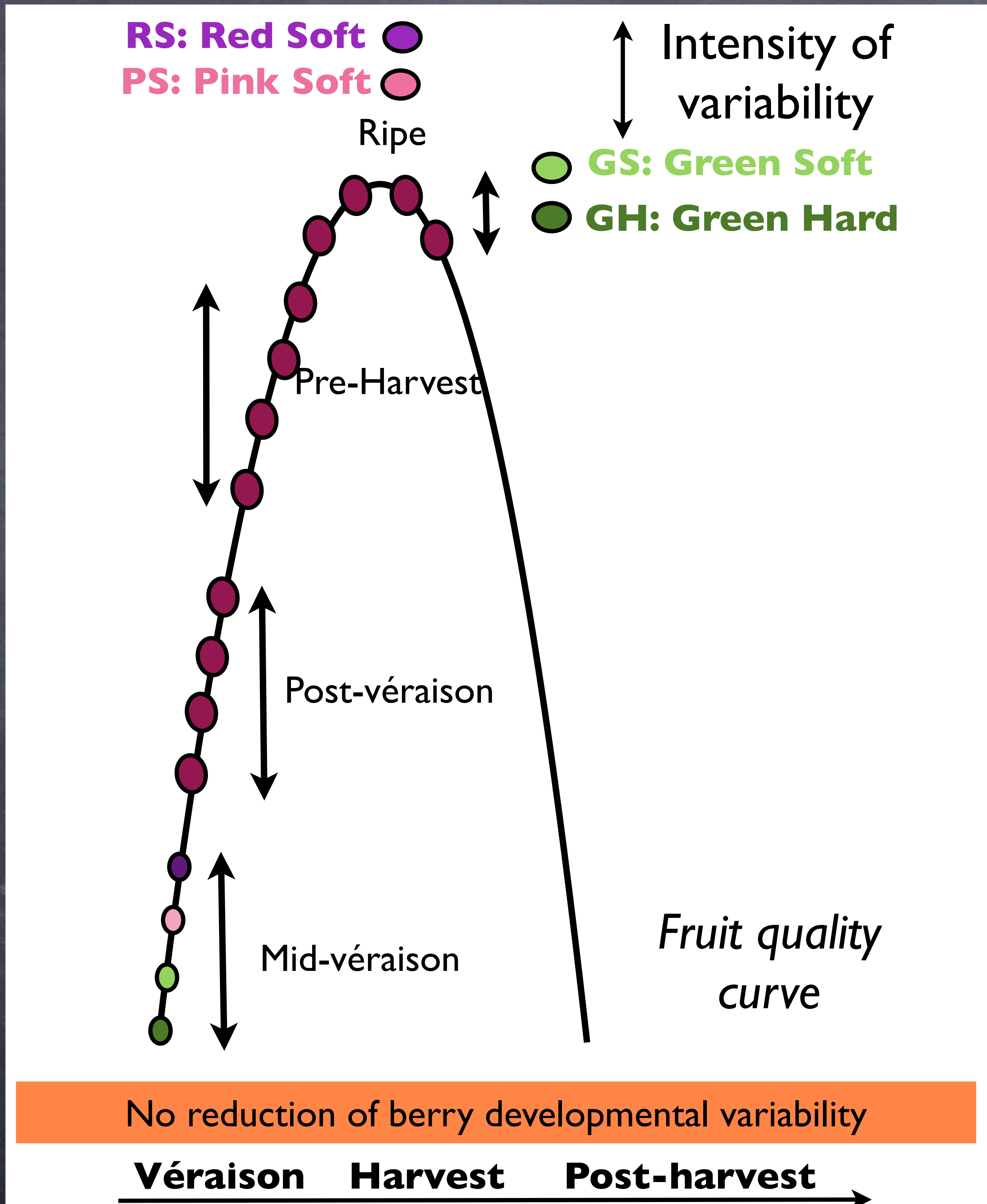

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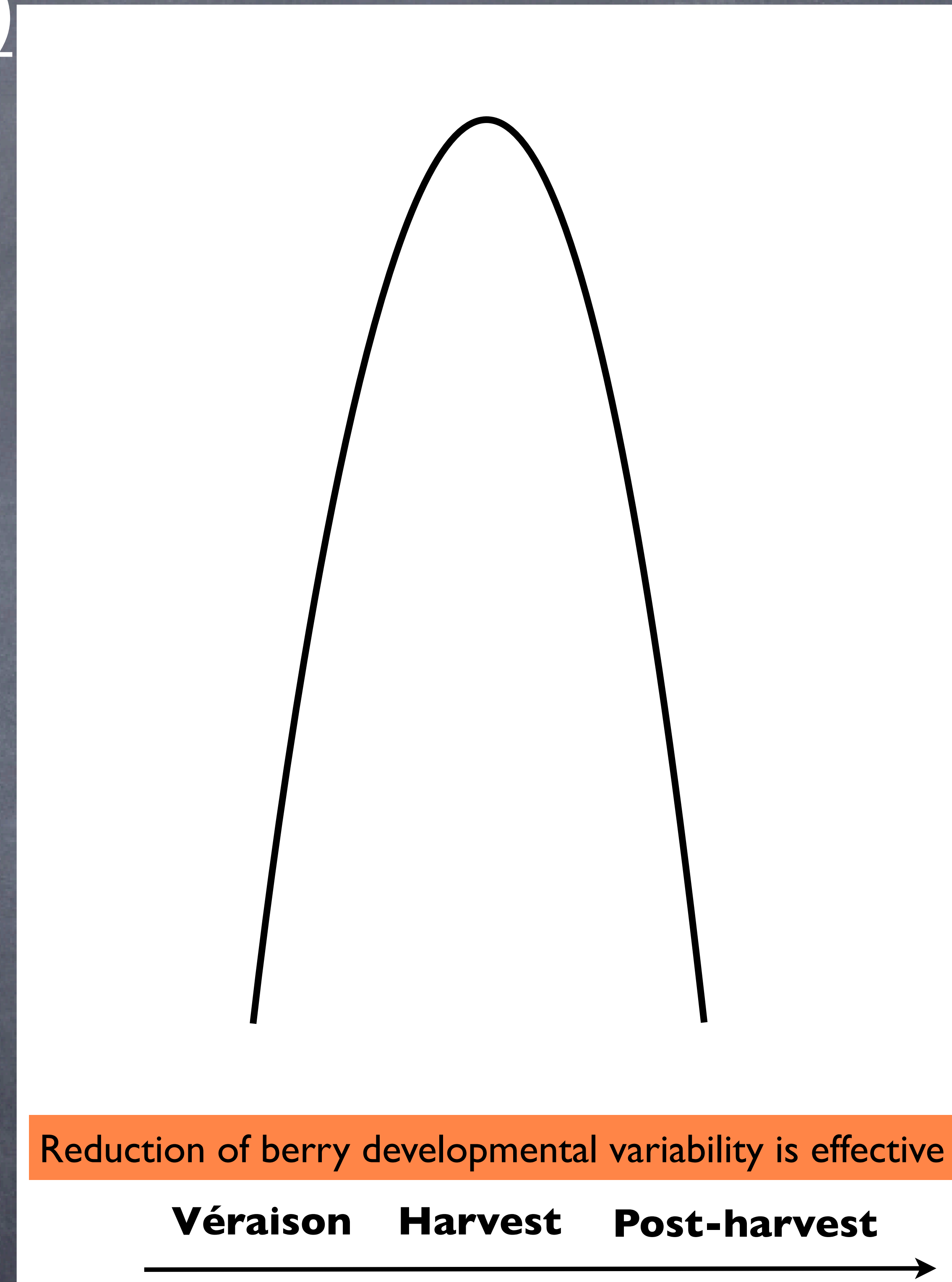

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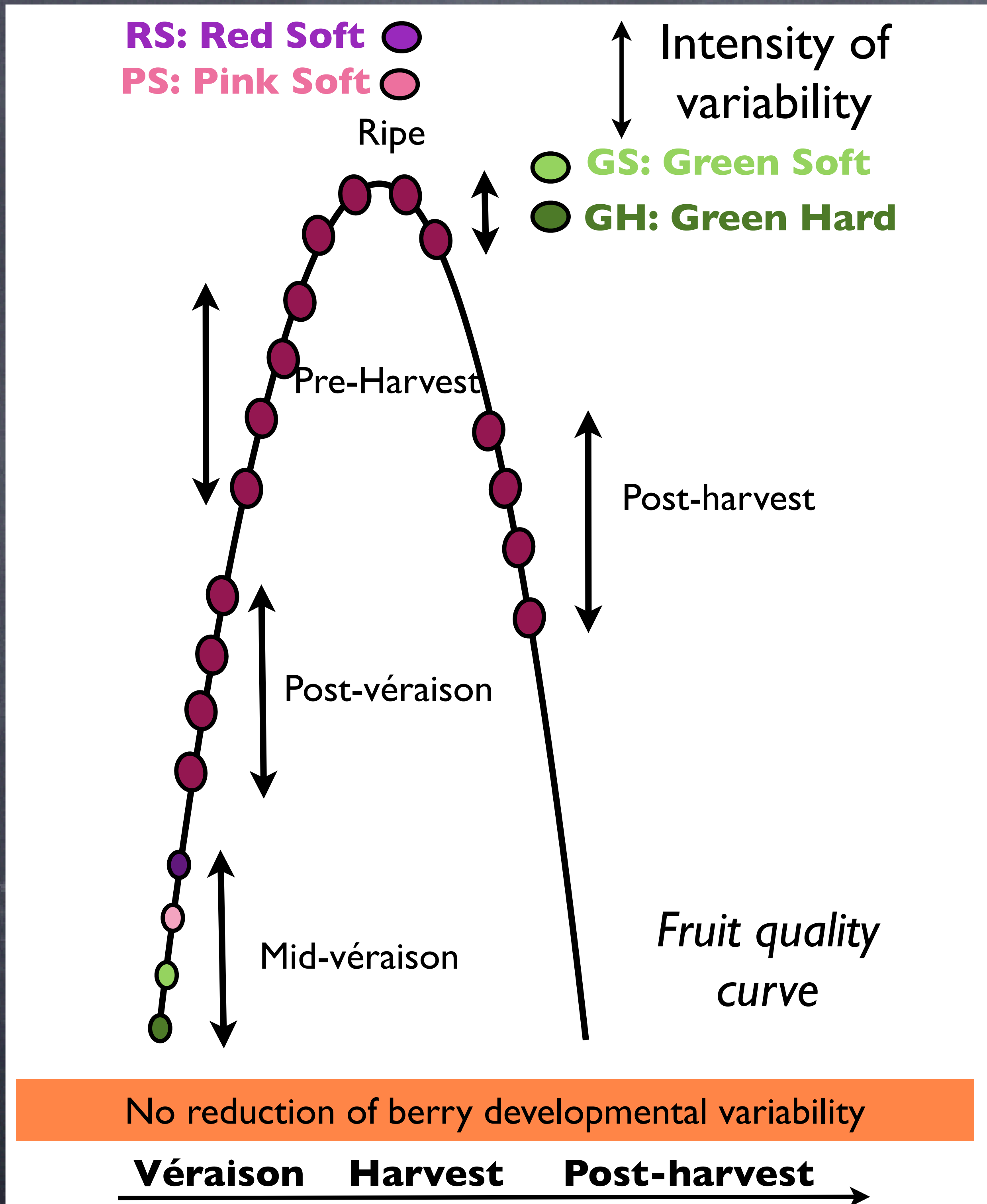

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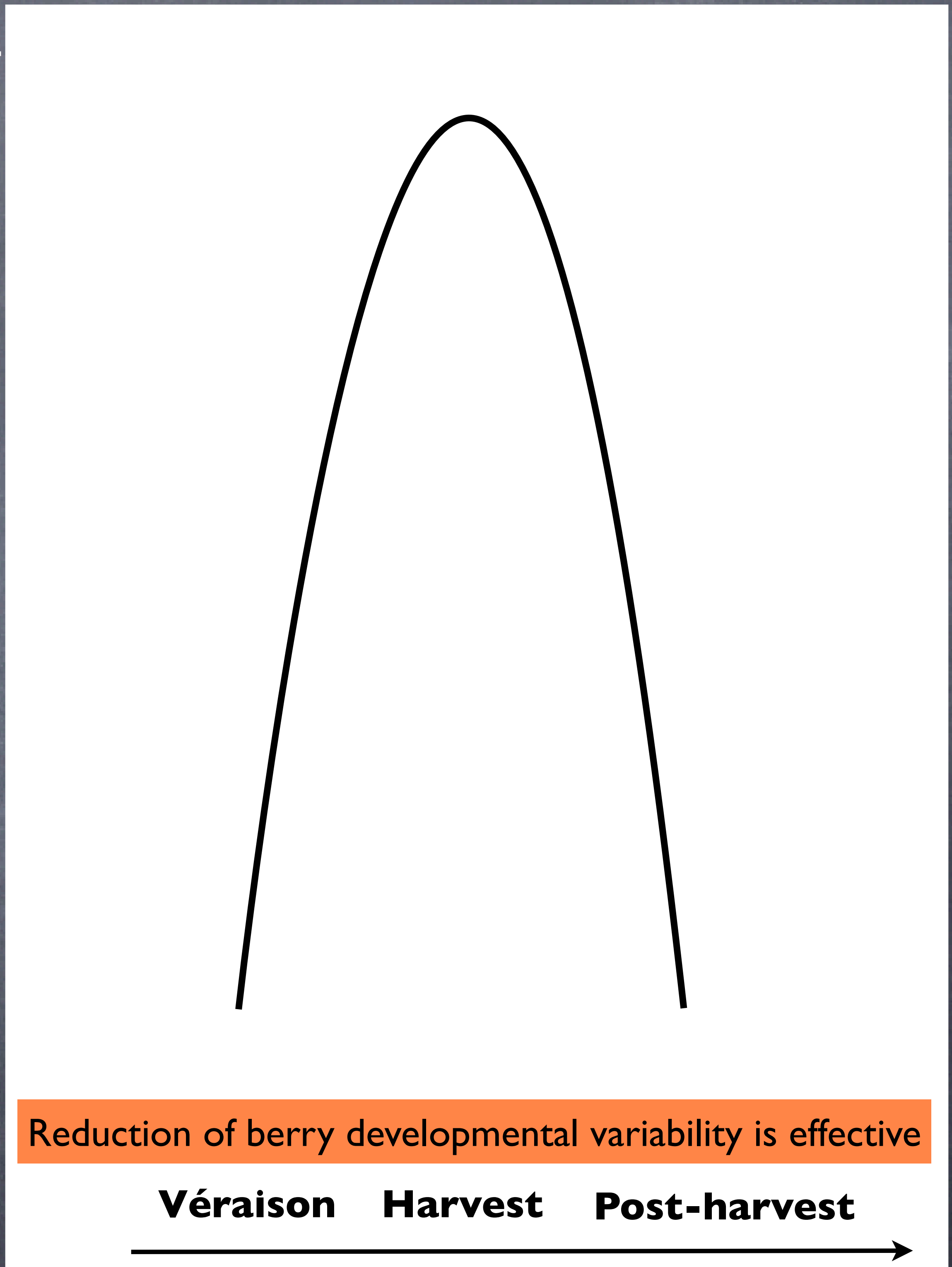

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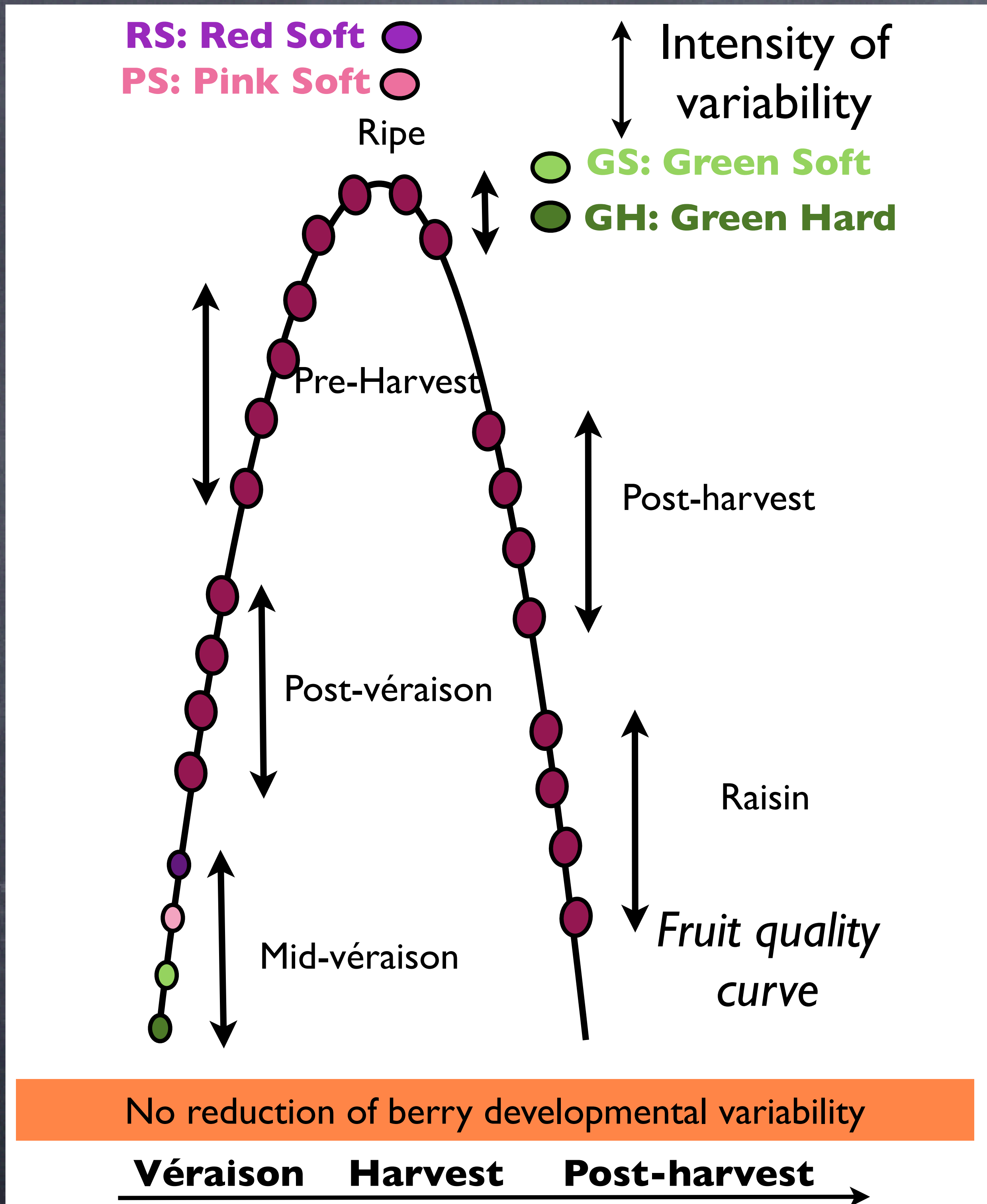

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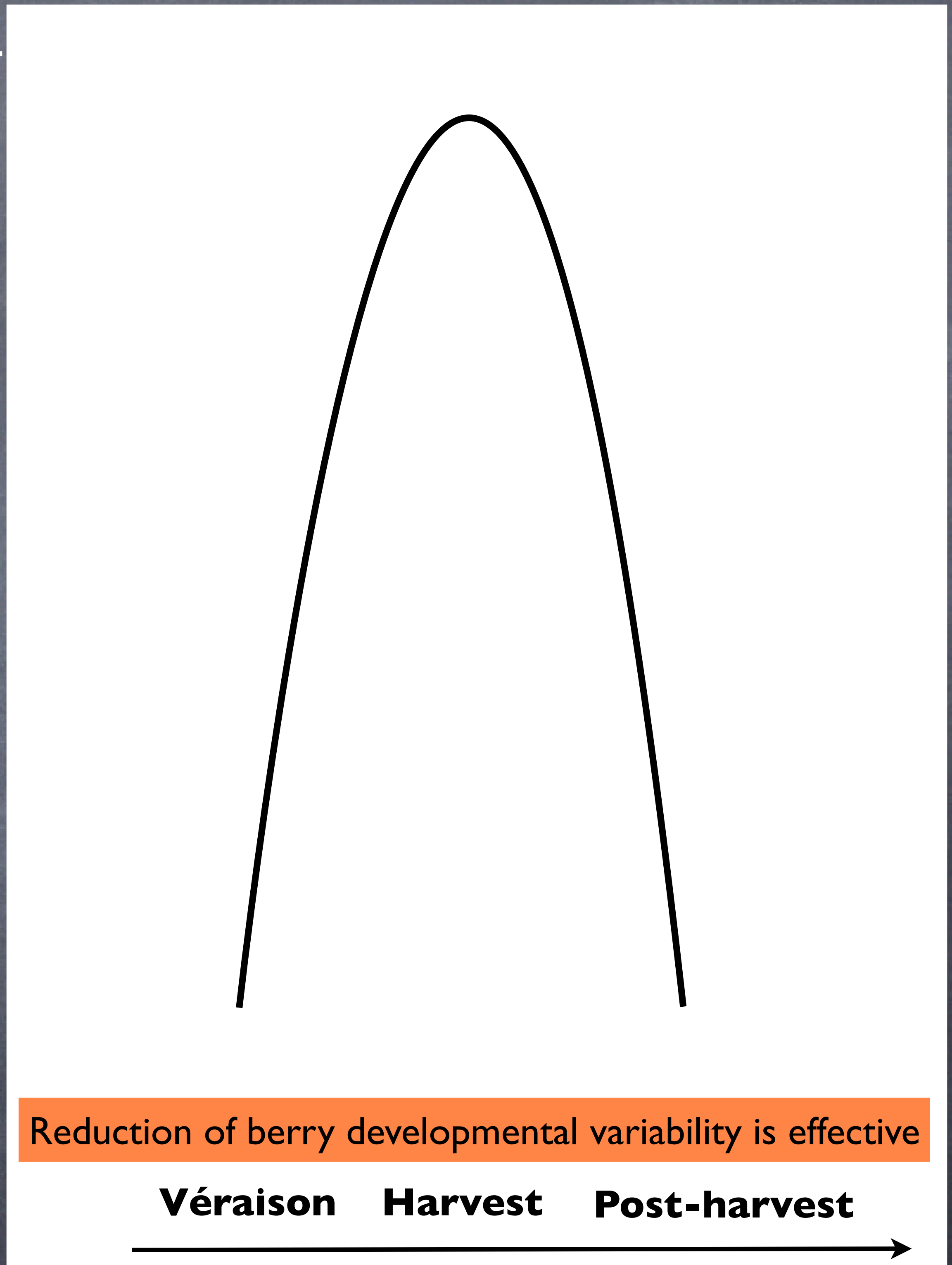

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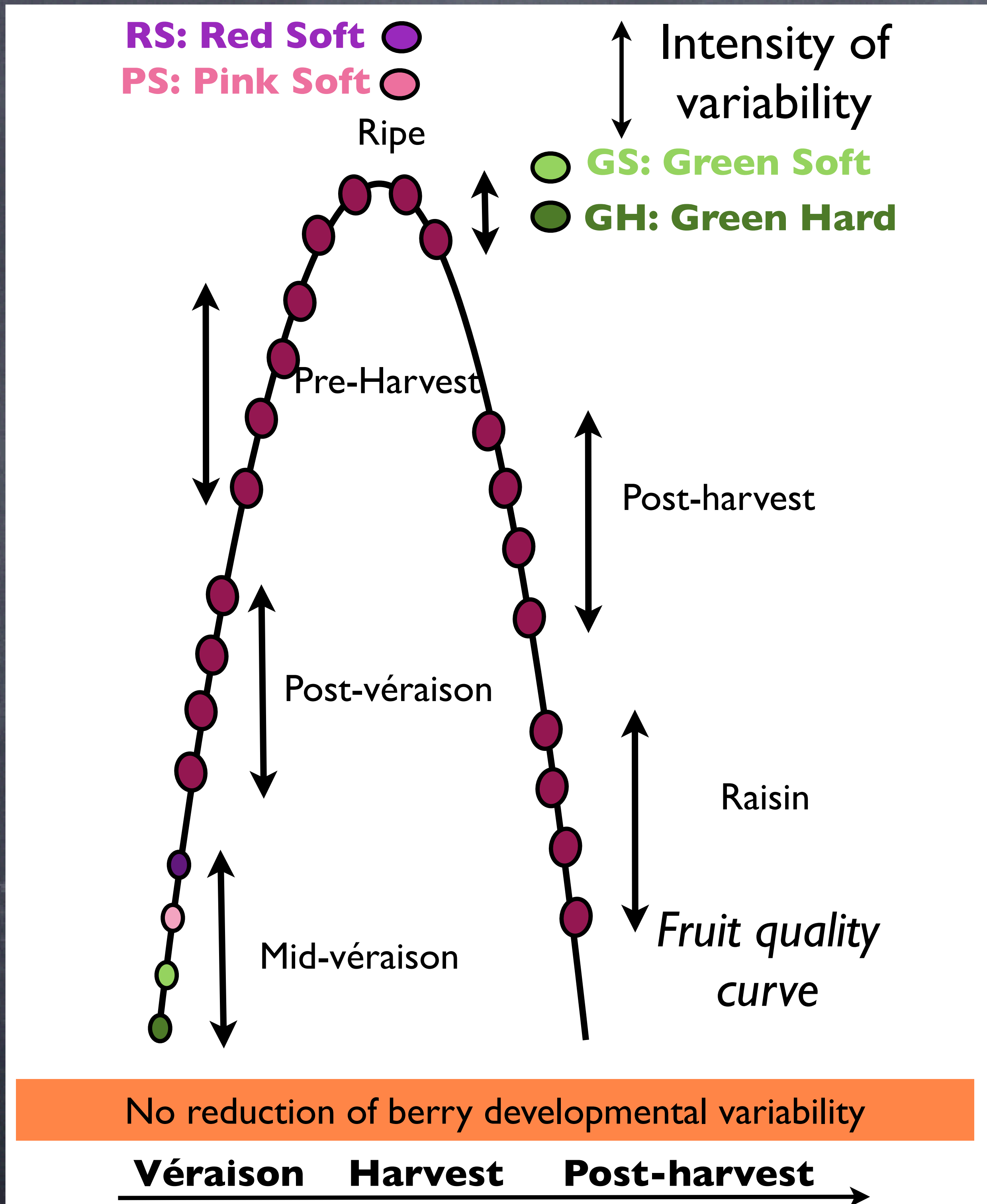

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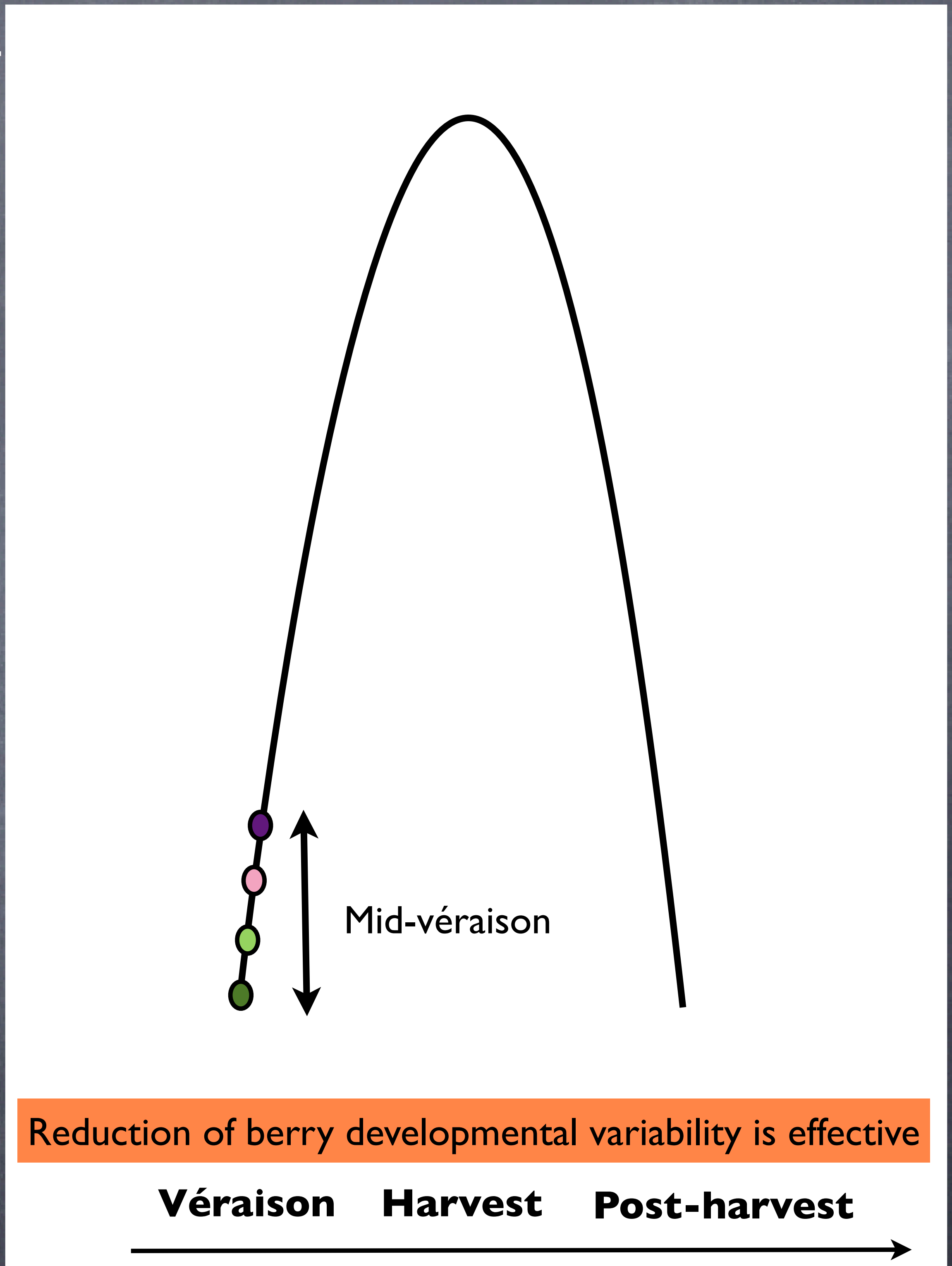

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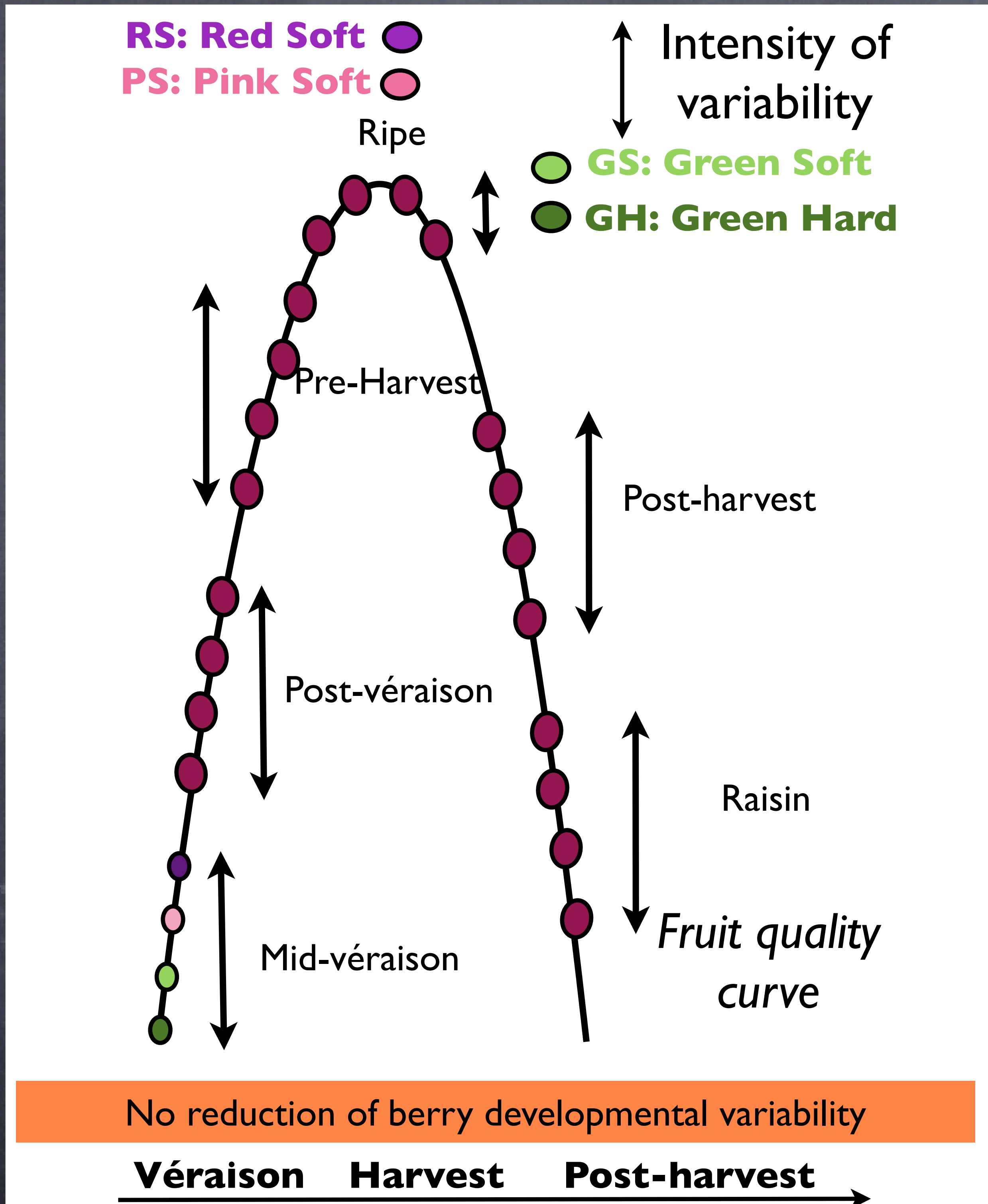

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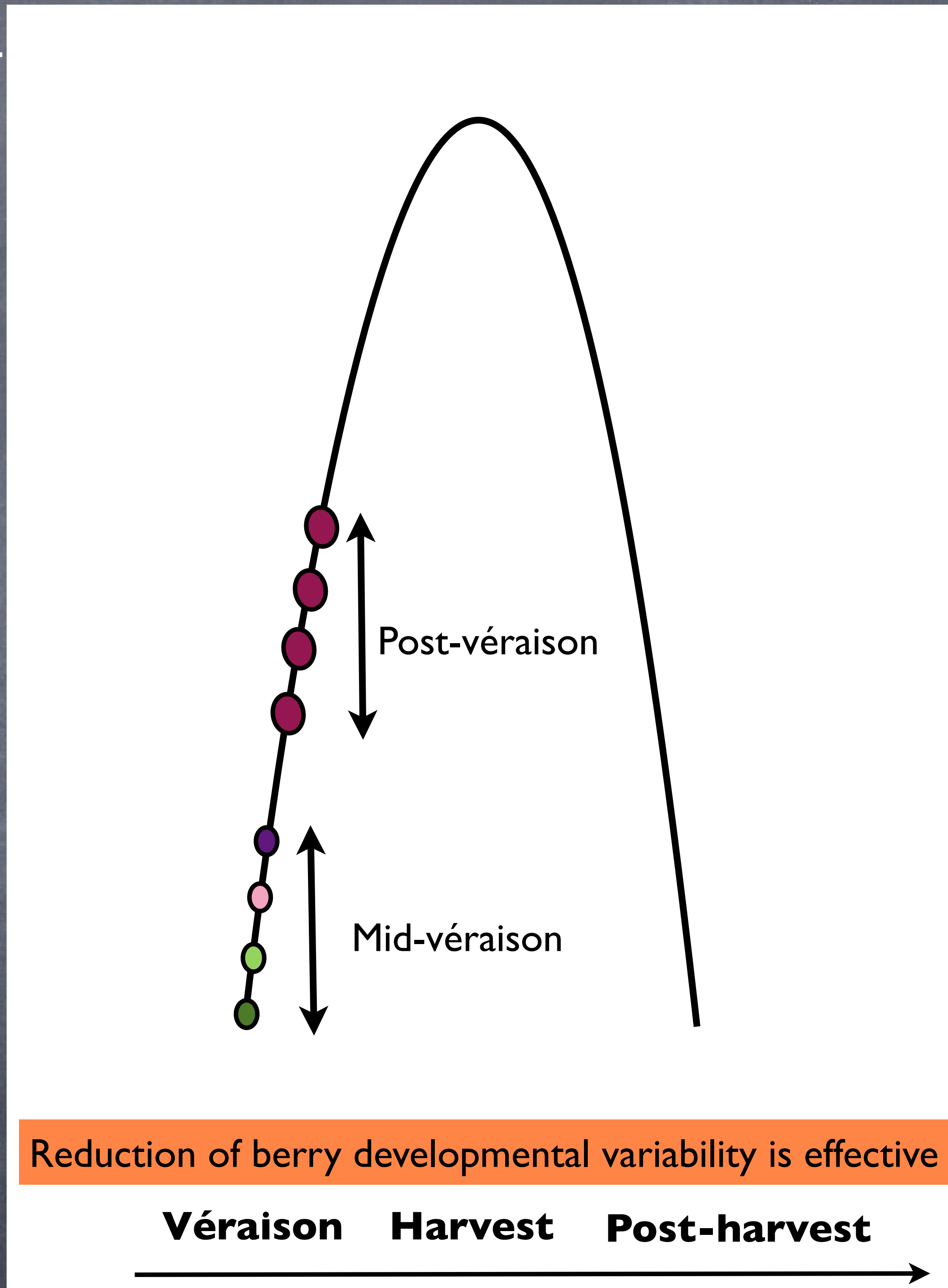

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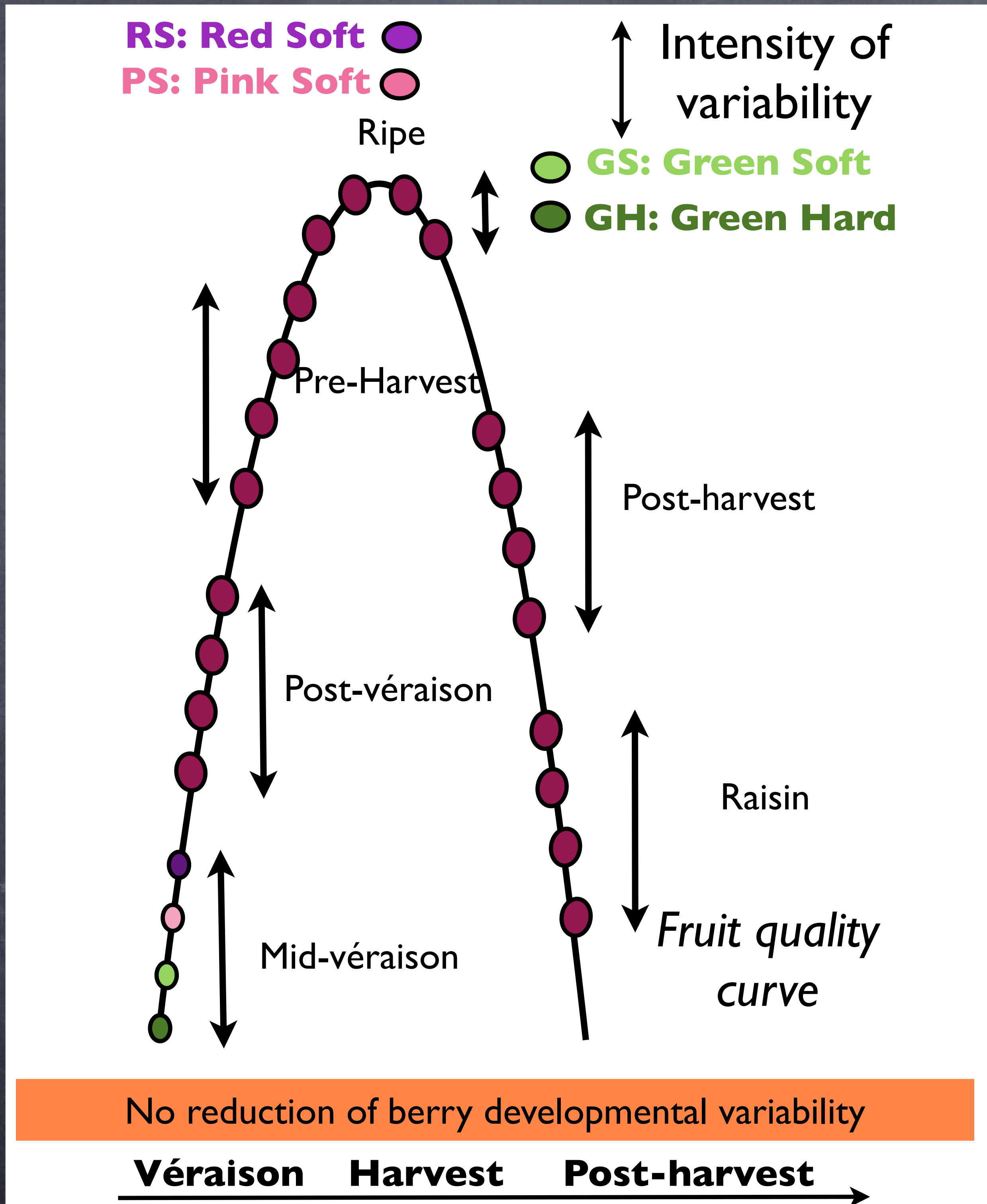

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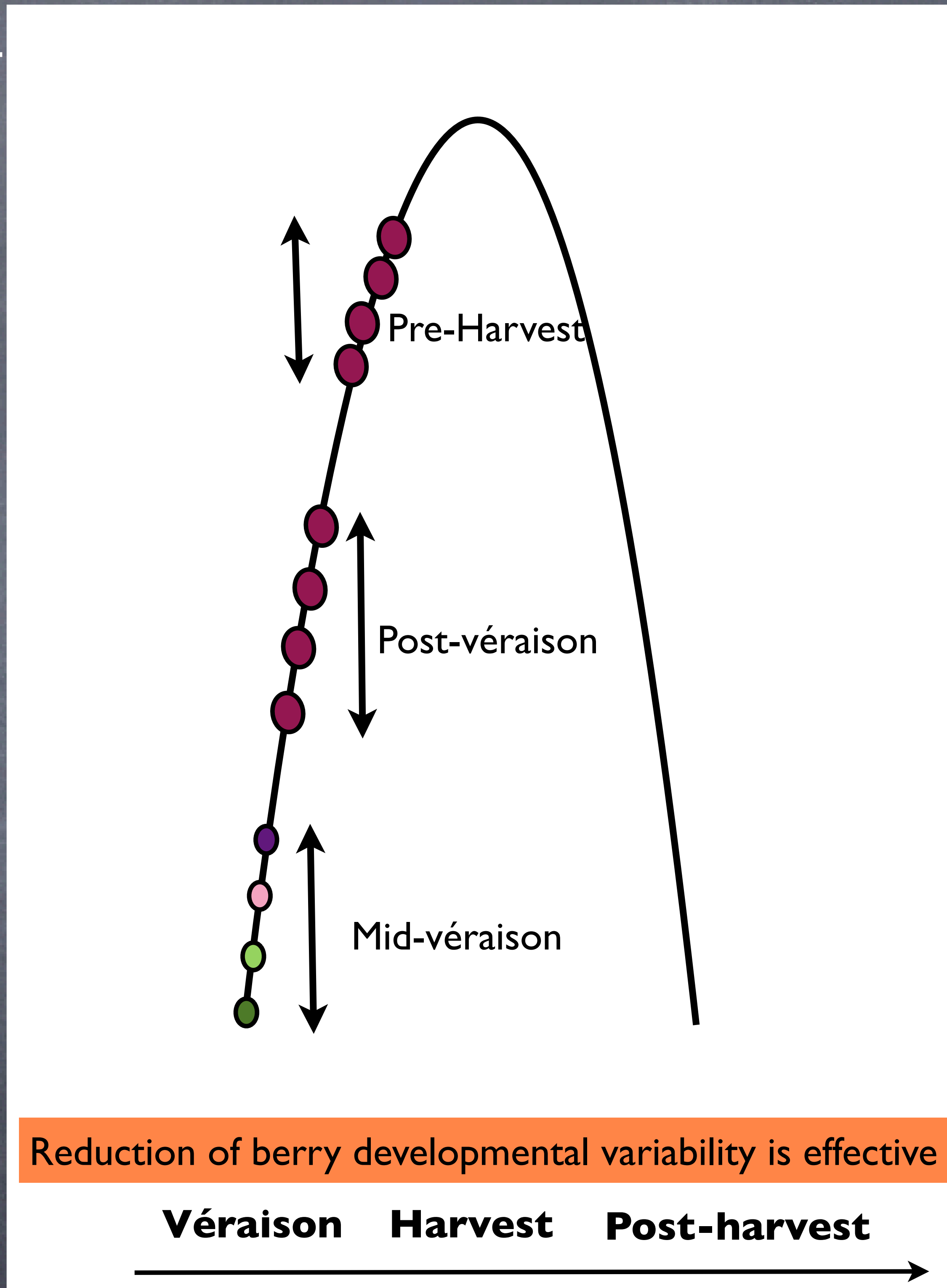

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Oregon Wine Research Institute
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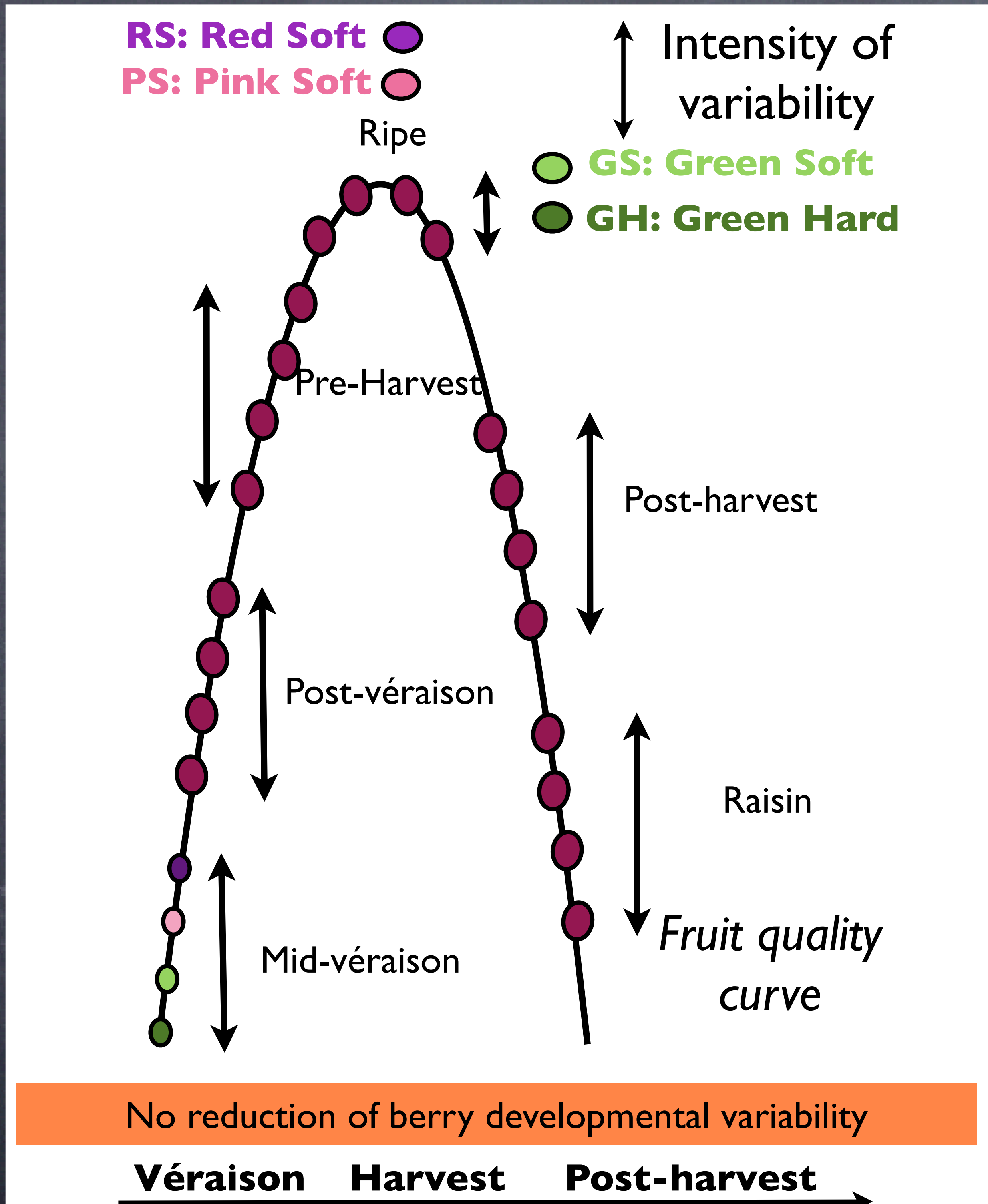

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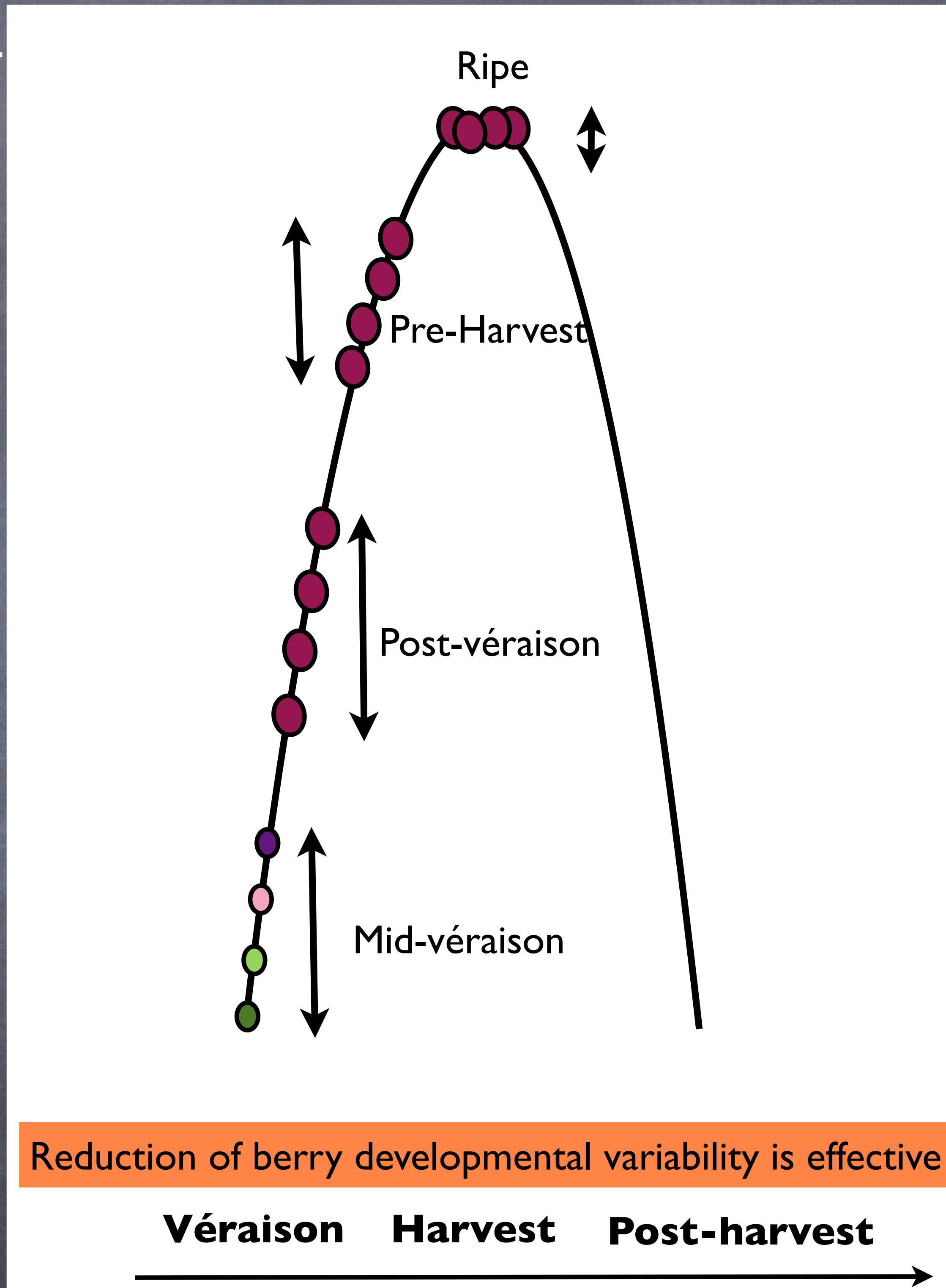

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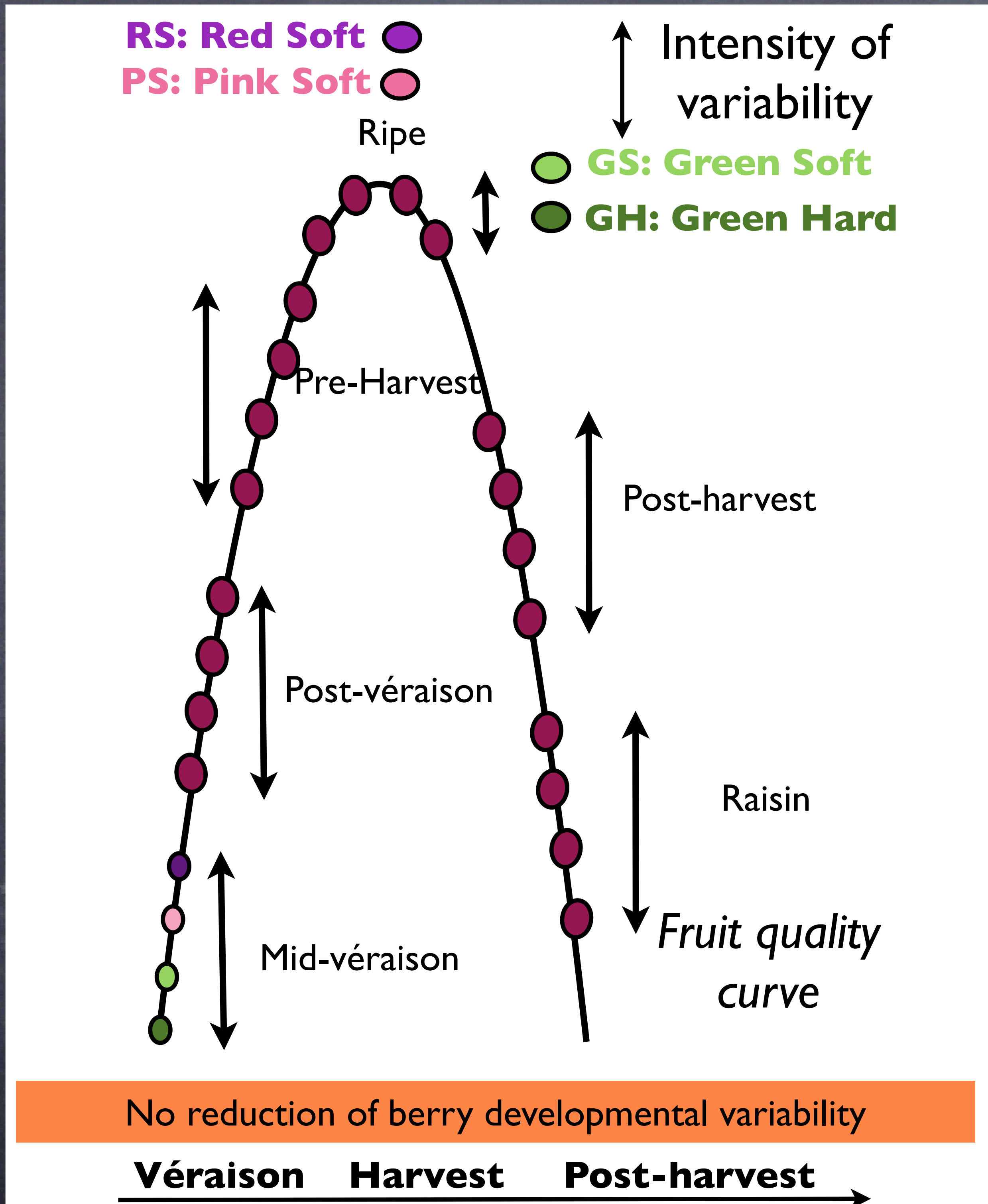

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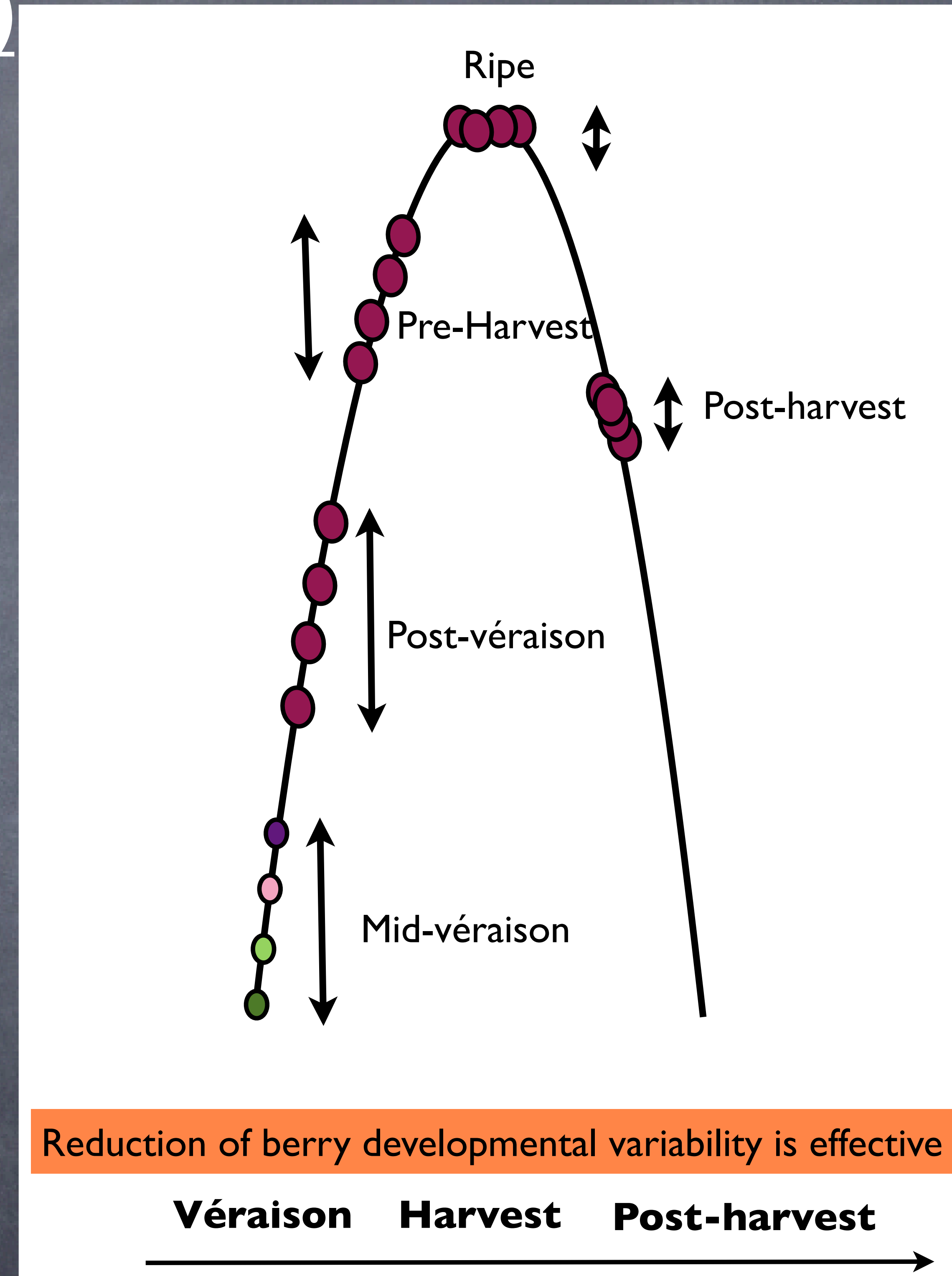

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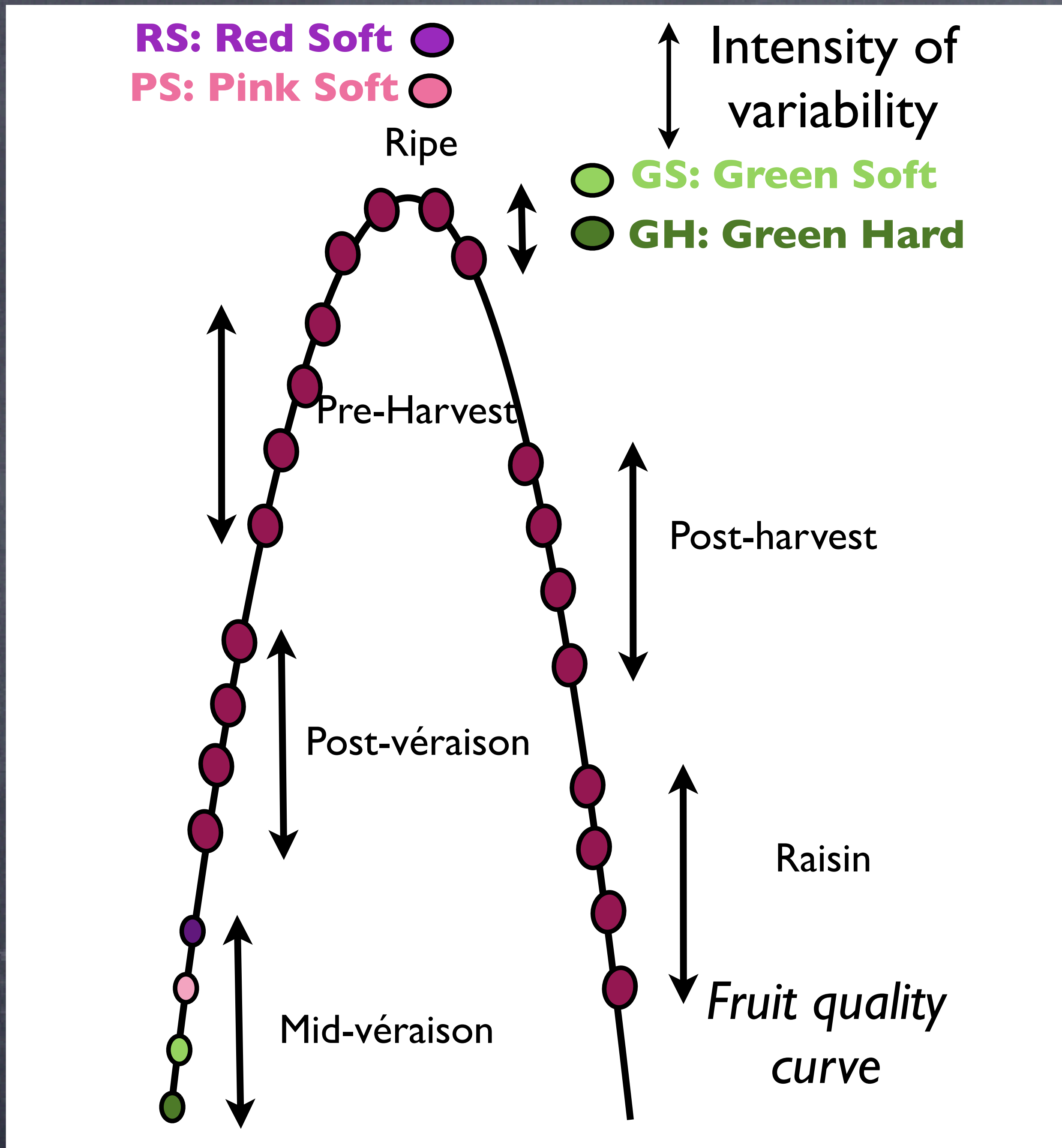
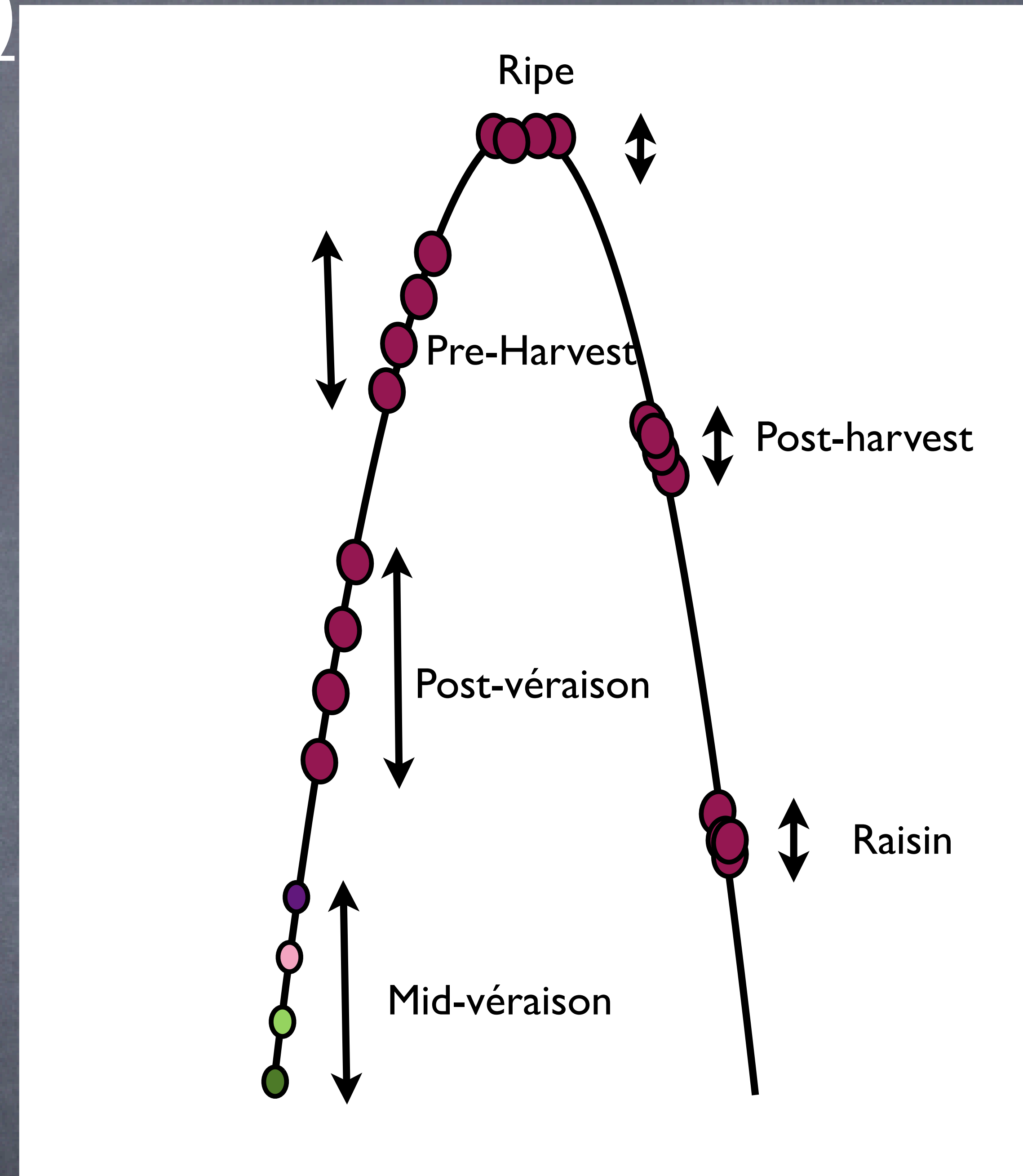


No reduction of berry developmental variability

2)



Reduction of berry developmental variability is effective

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Reduction of berry class variability

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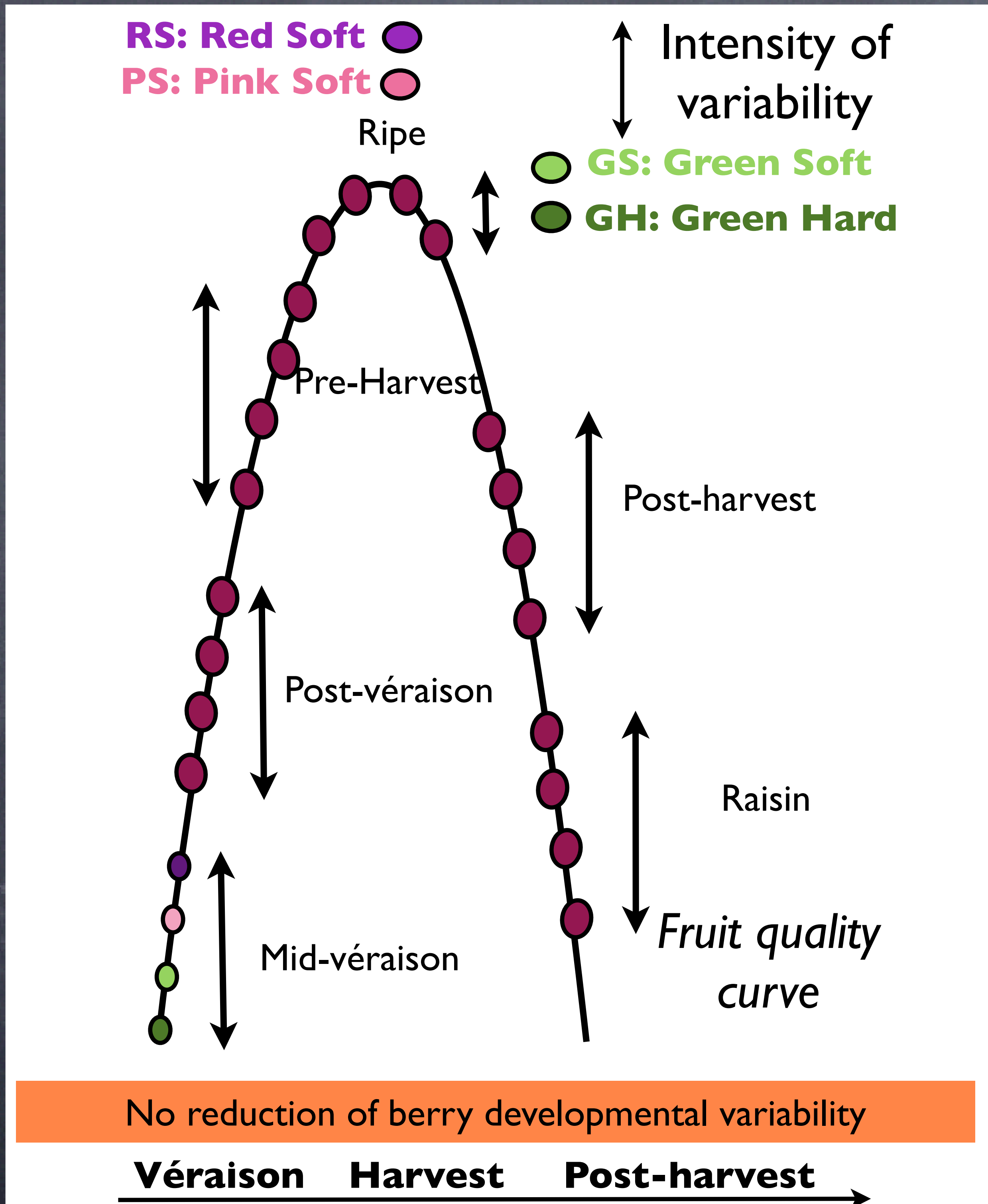

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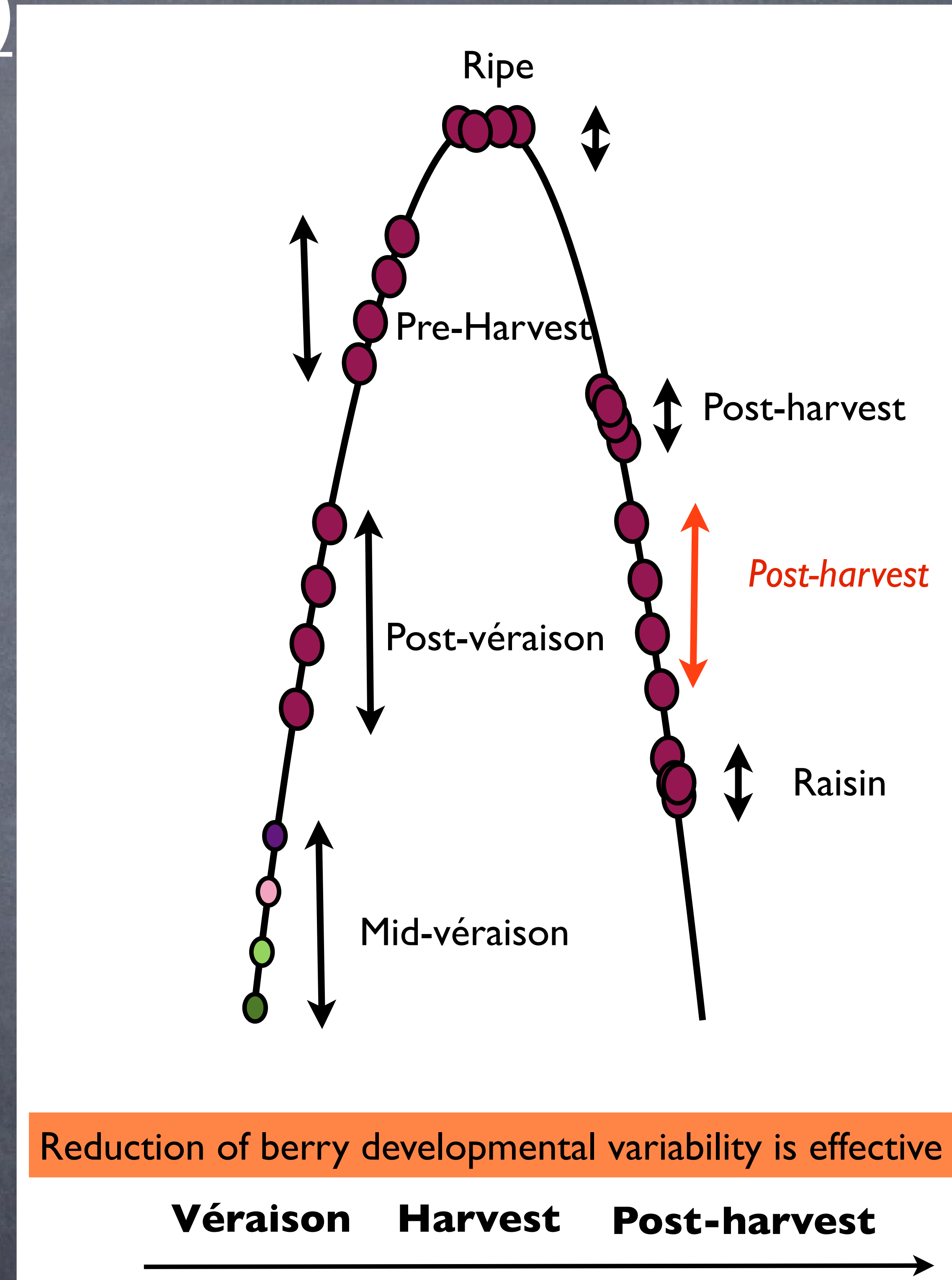

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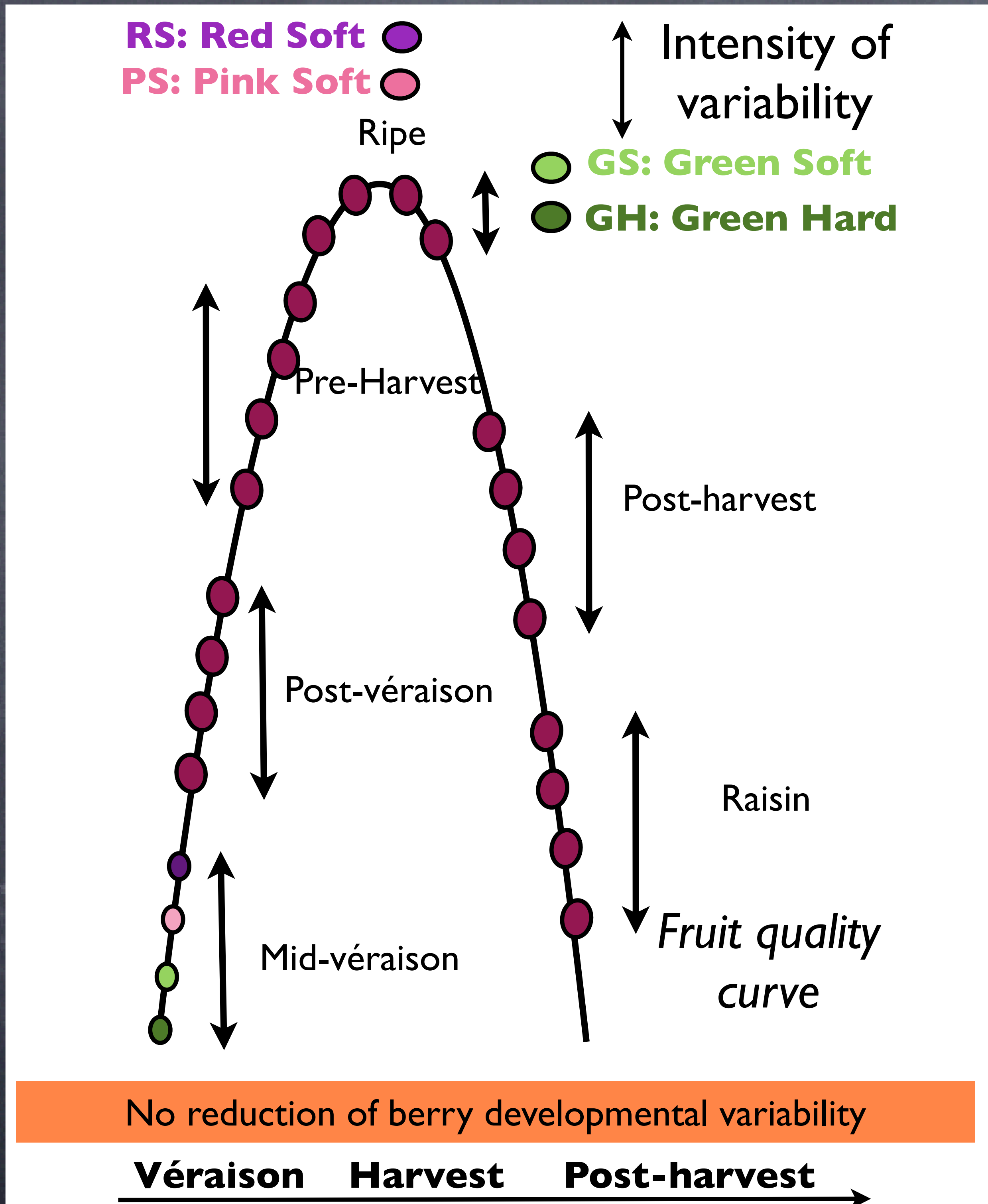

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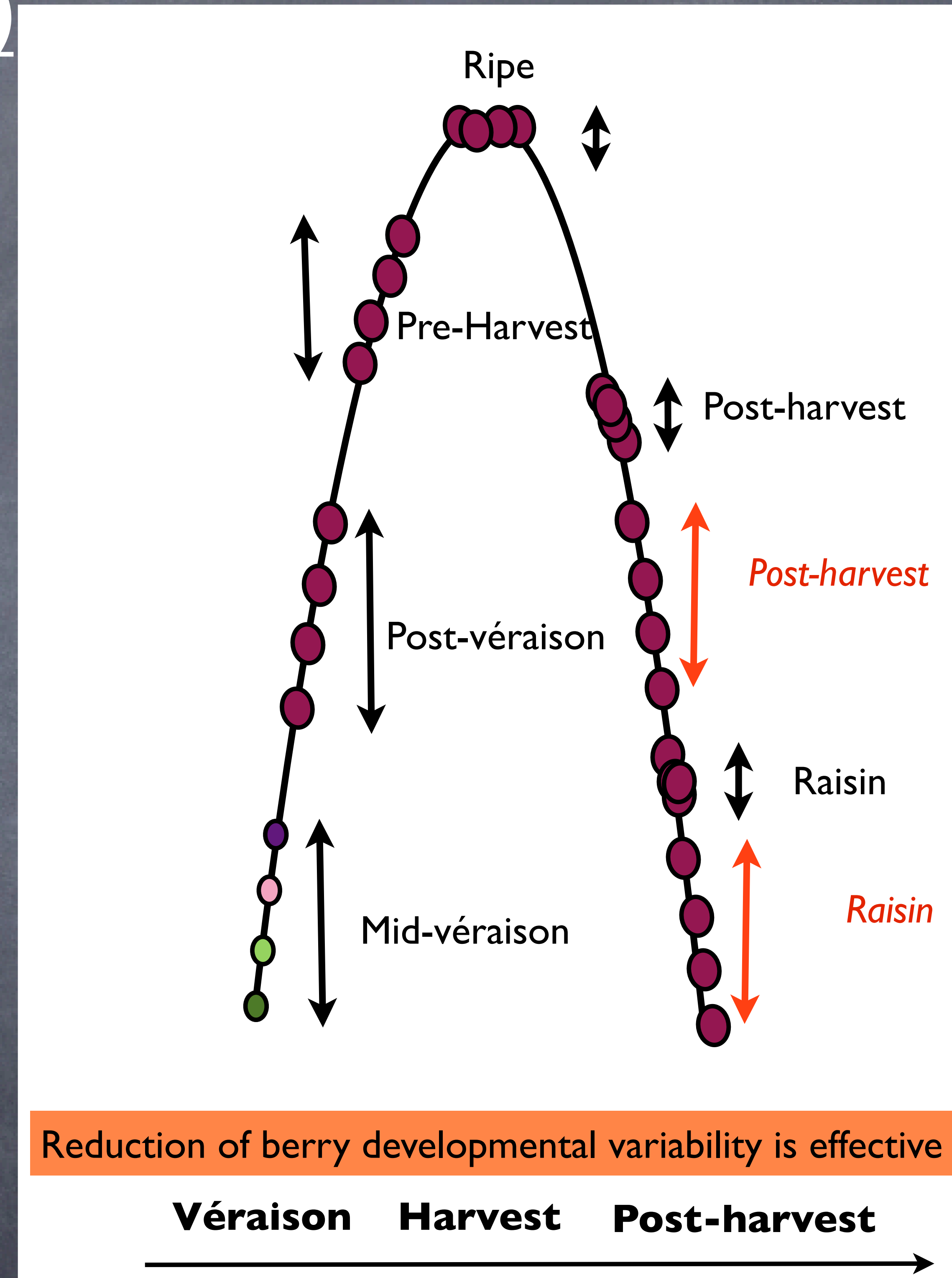

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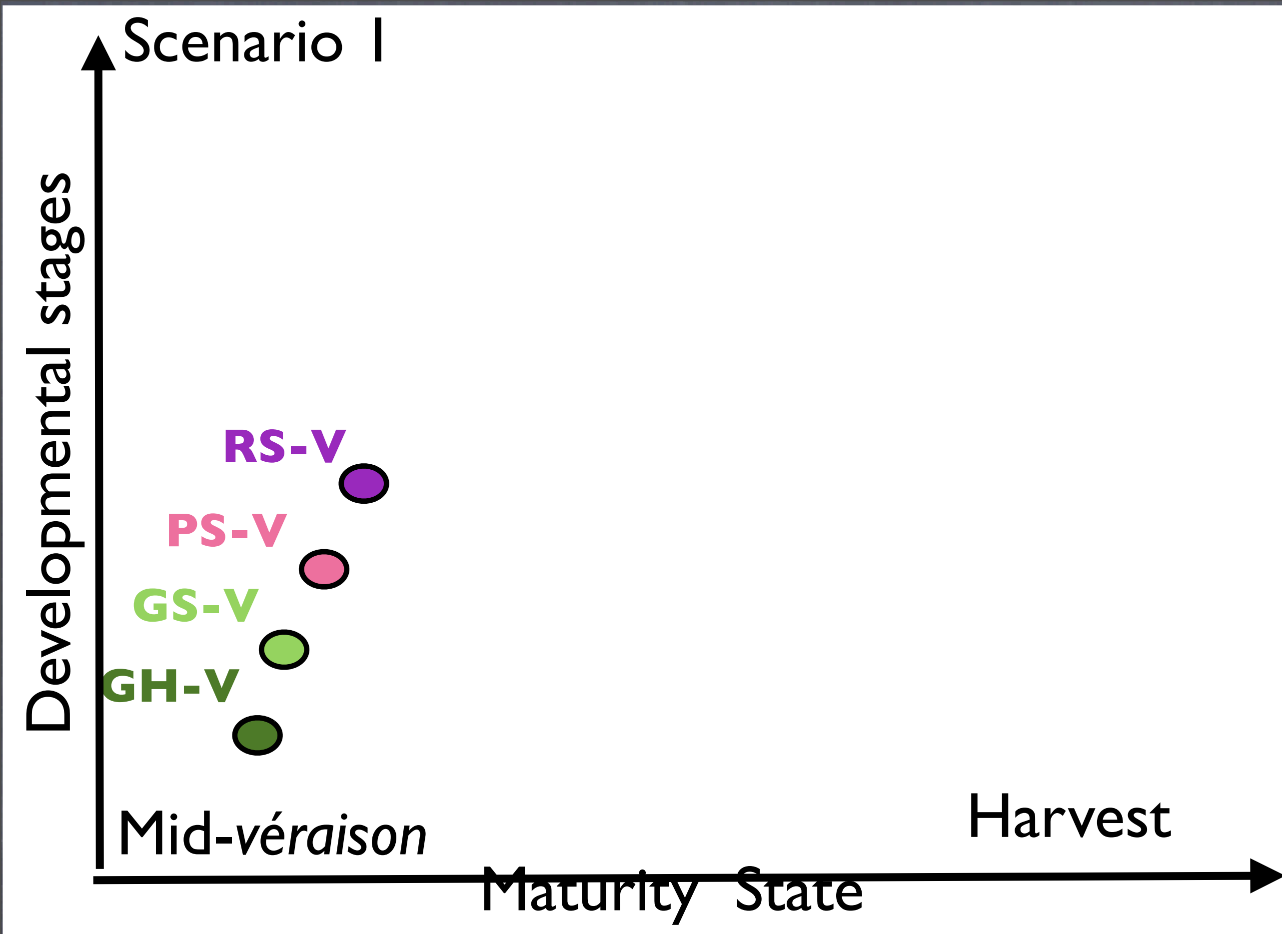
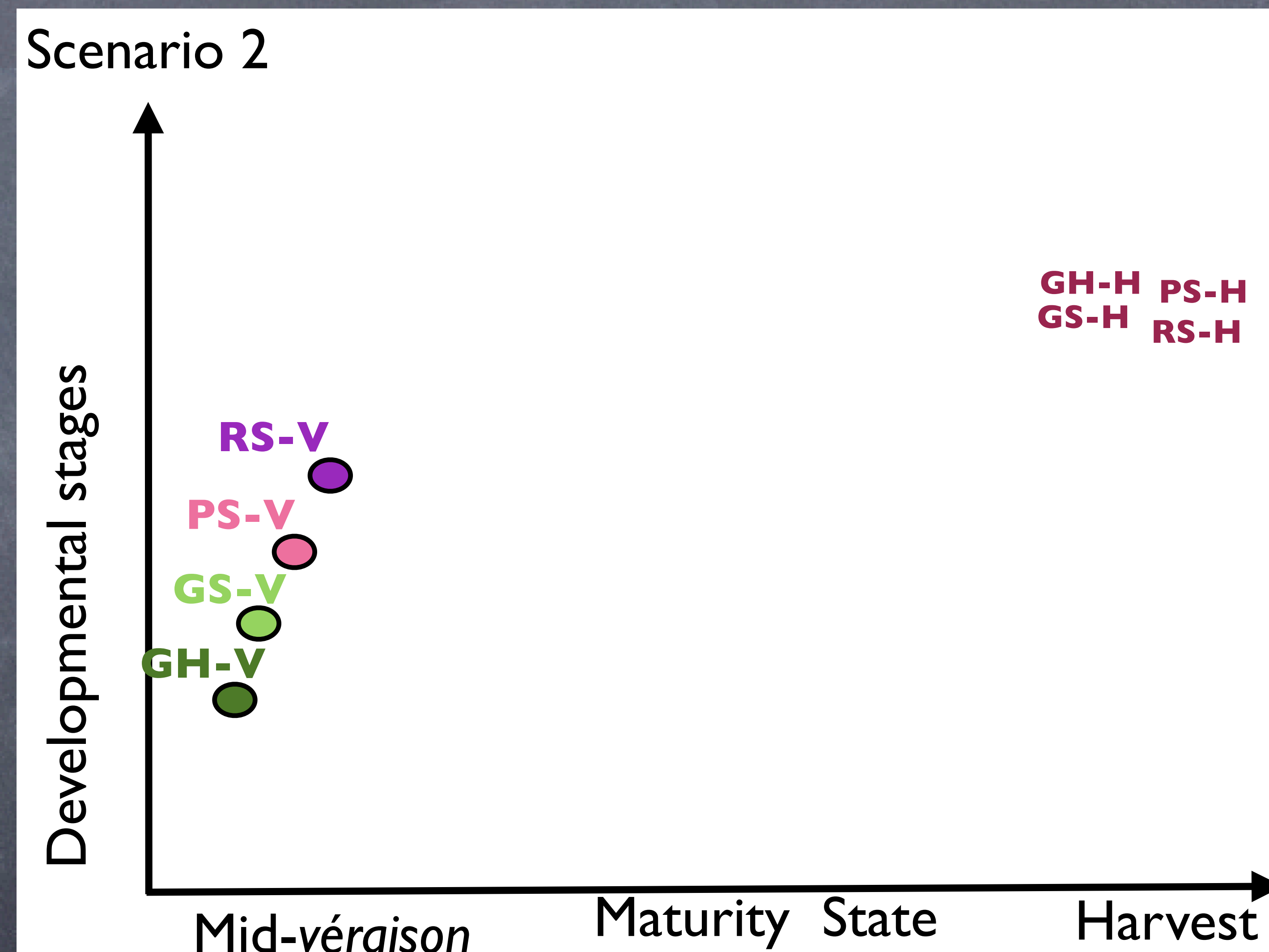


If we assume differences at mid-*véraison*, we must still see differences near harvest at the developmental level



If under ripe berries are catching up we do not know when that happens....

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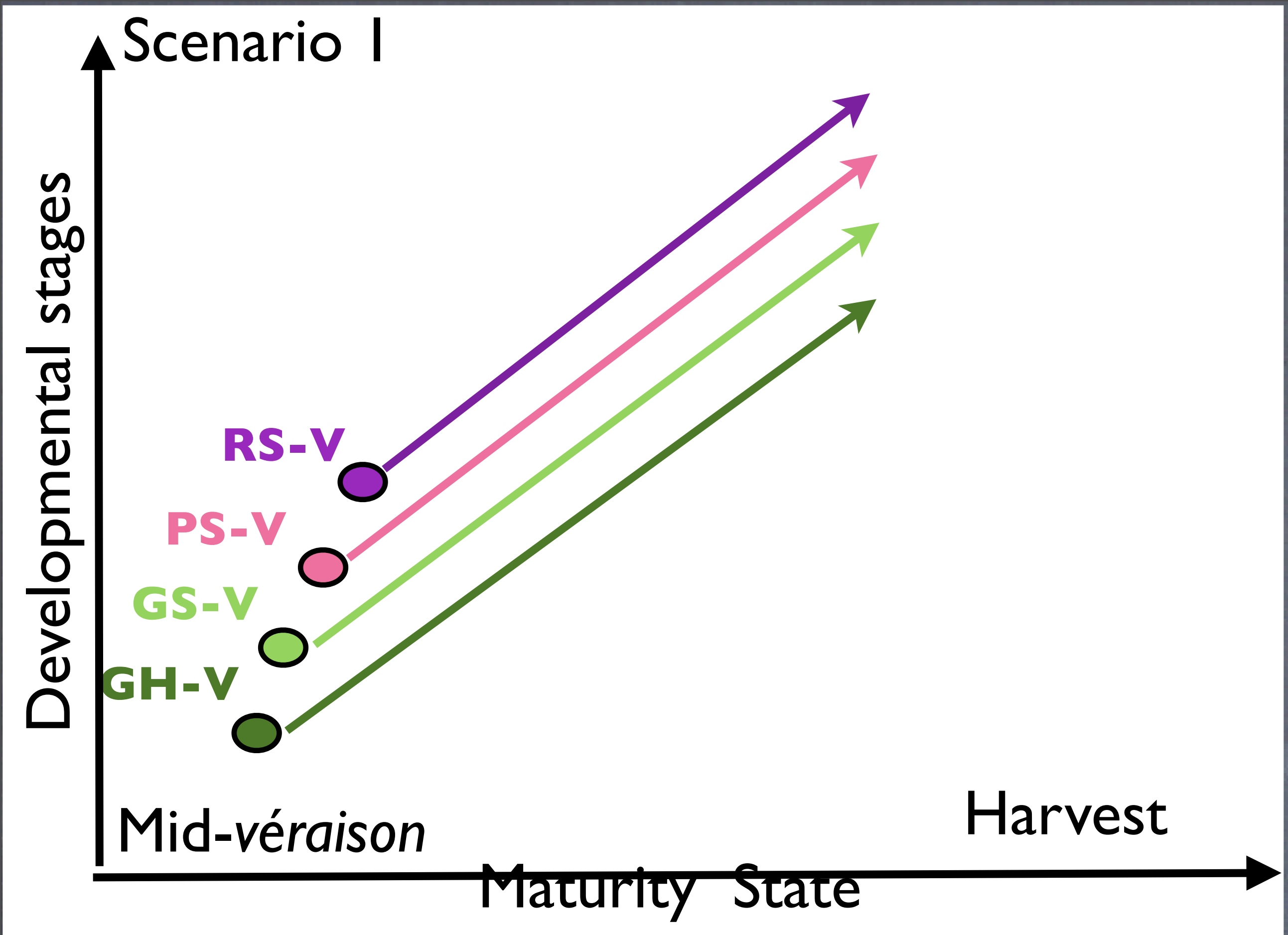
Oregon Wine Research Institute
Oregon State University



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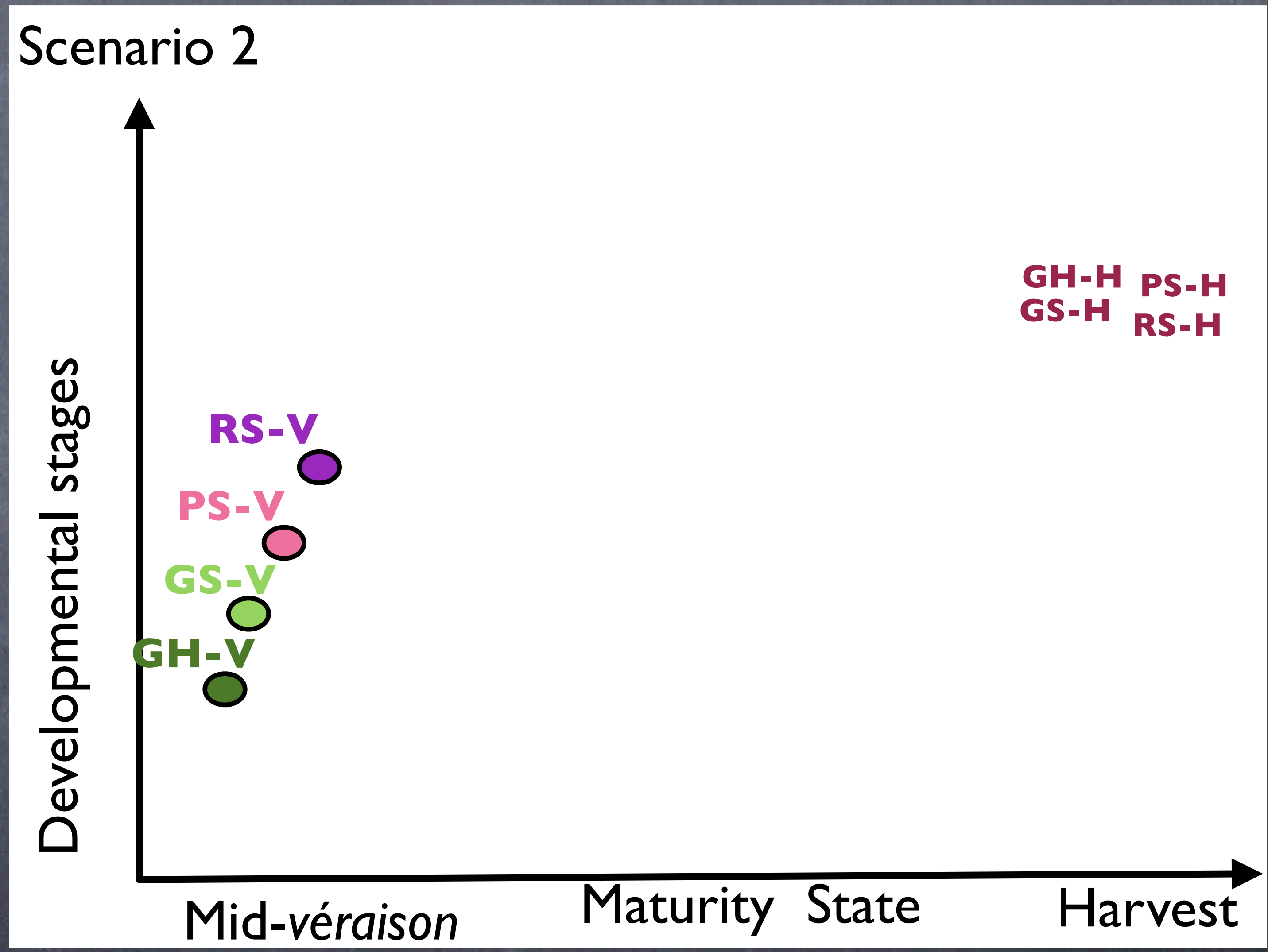

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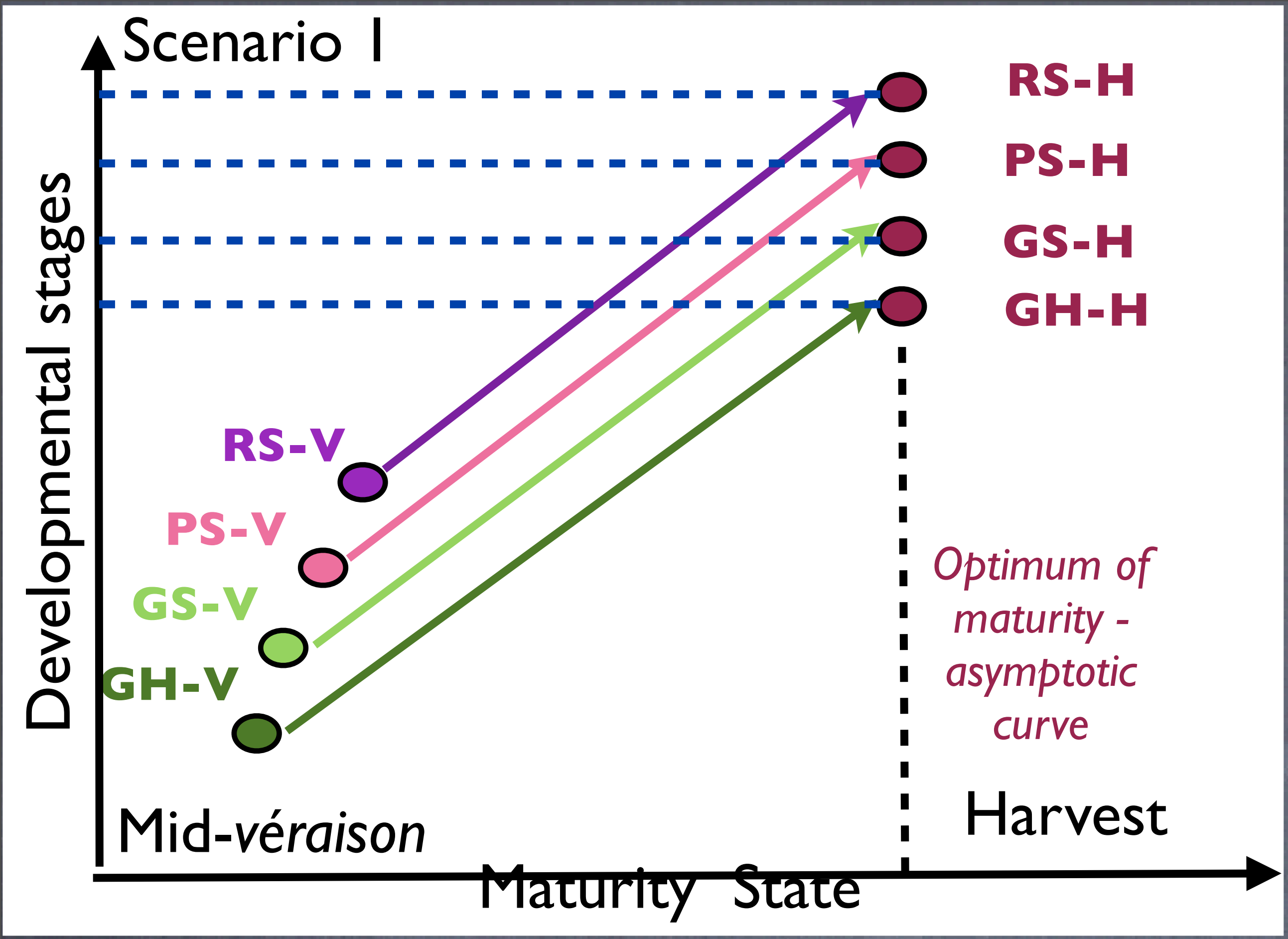

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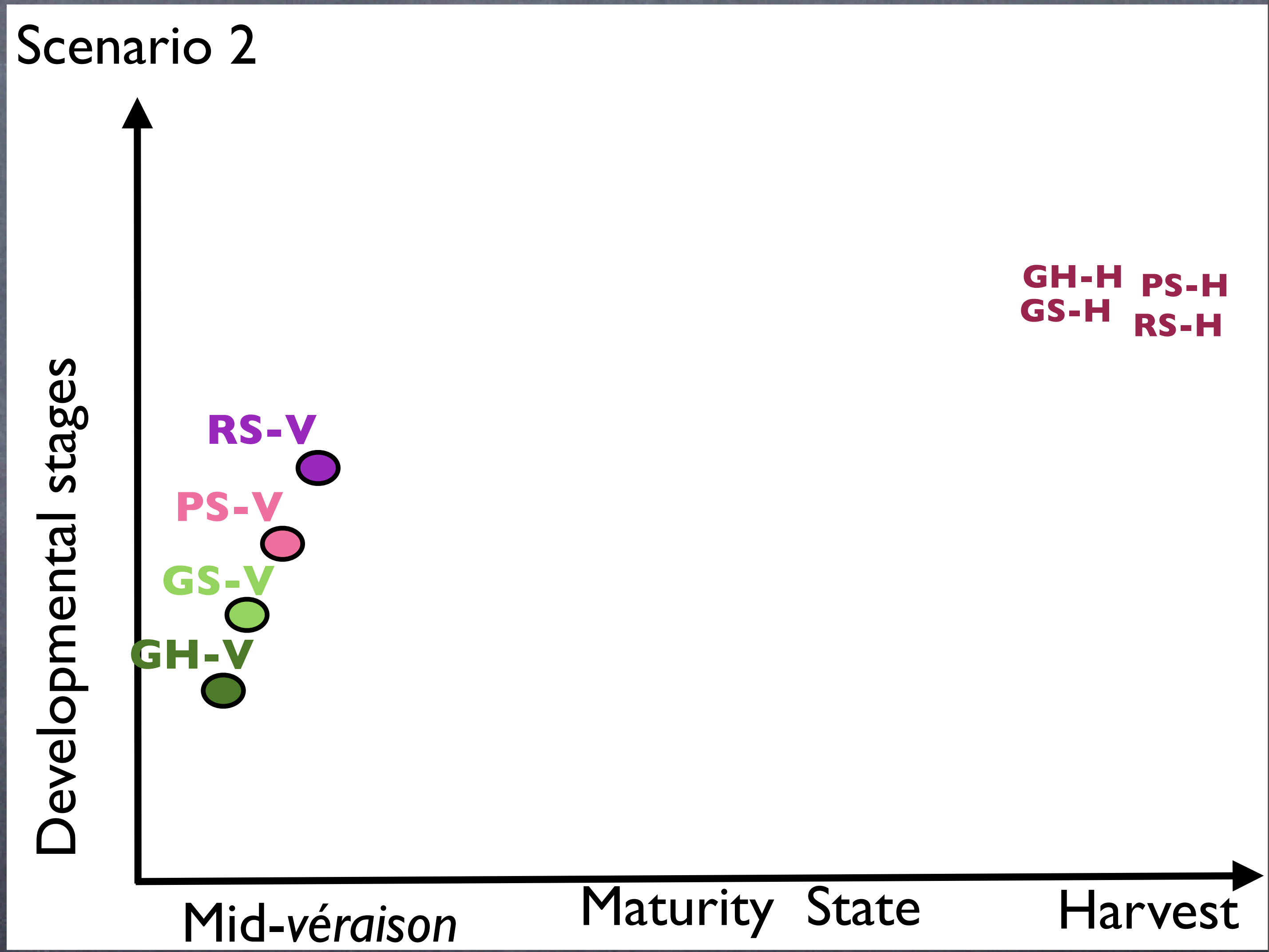

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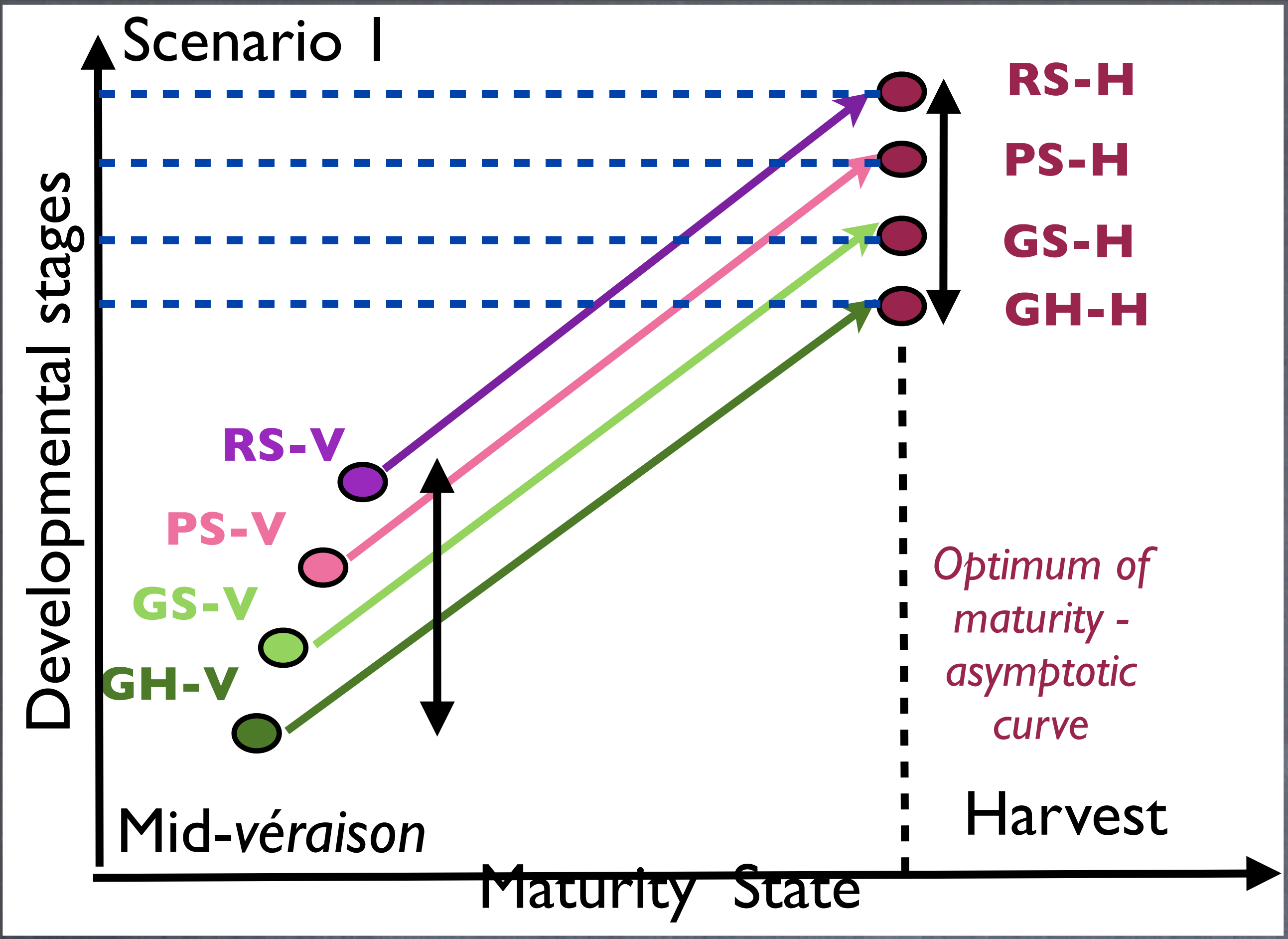

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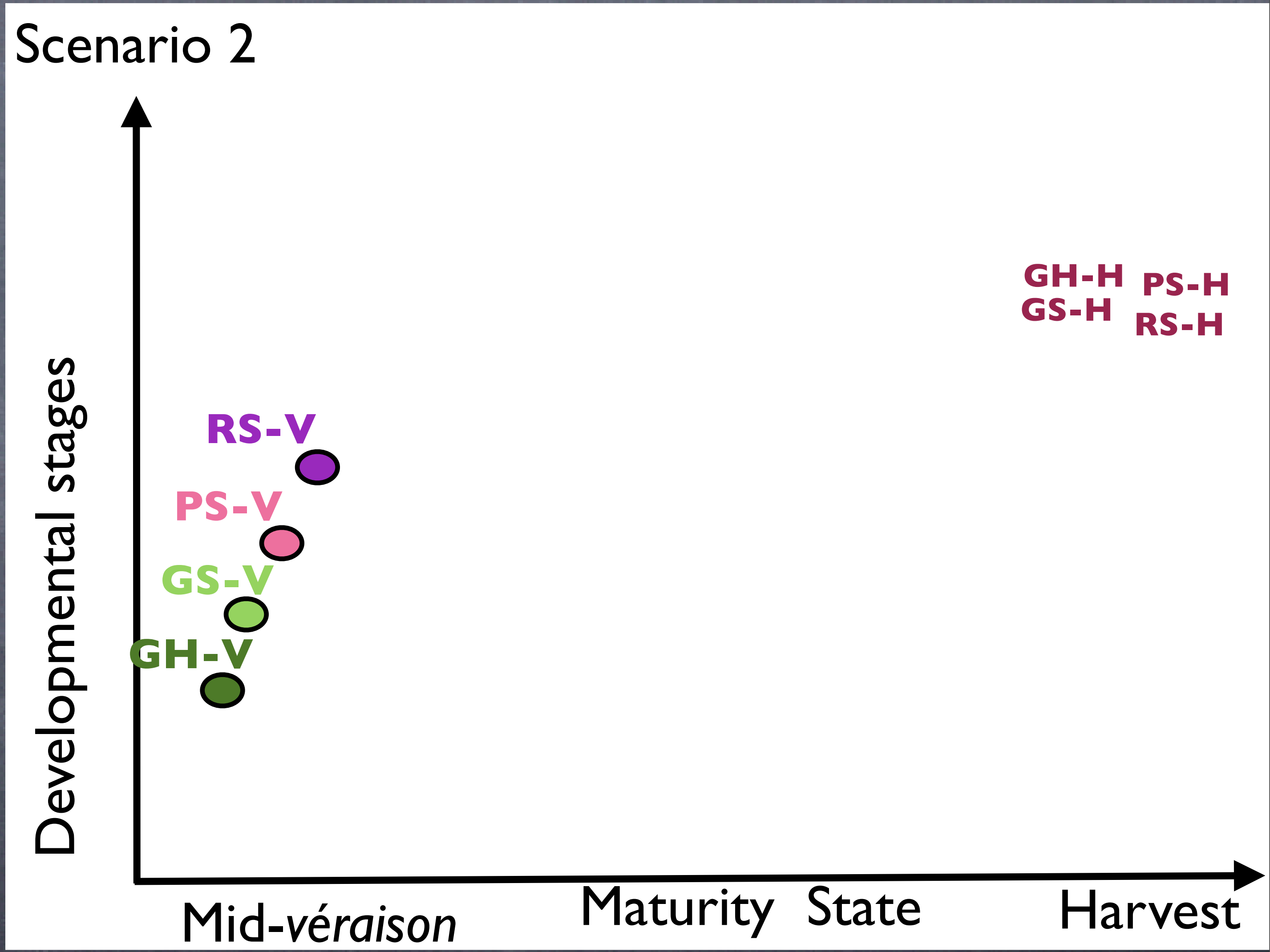

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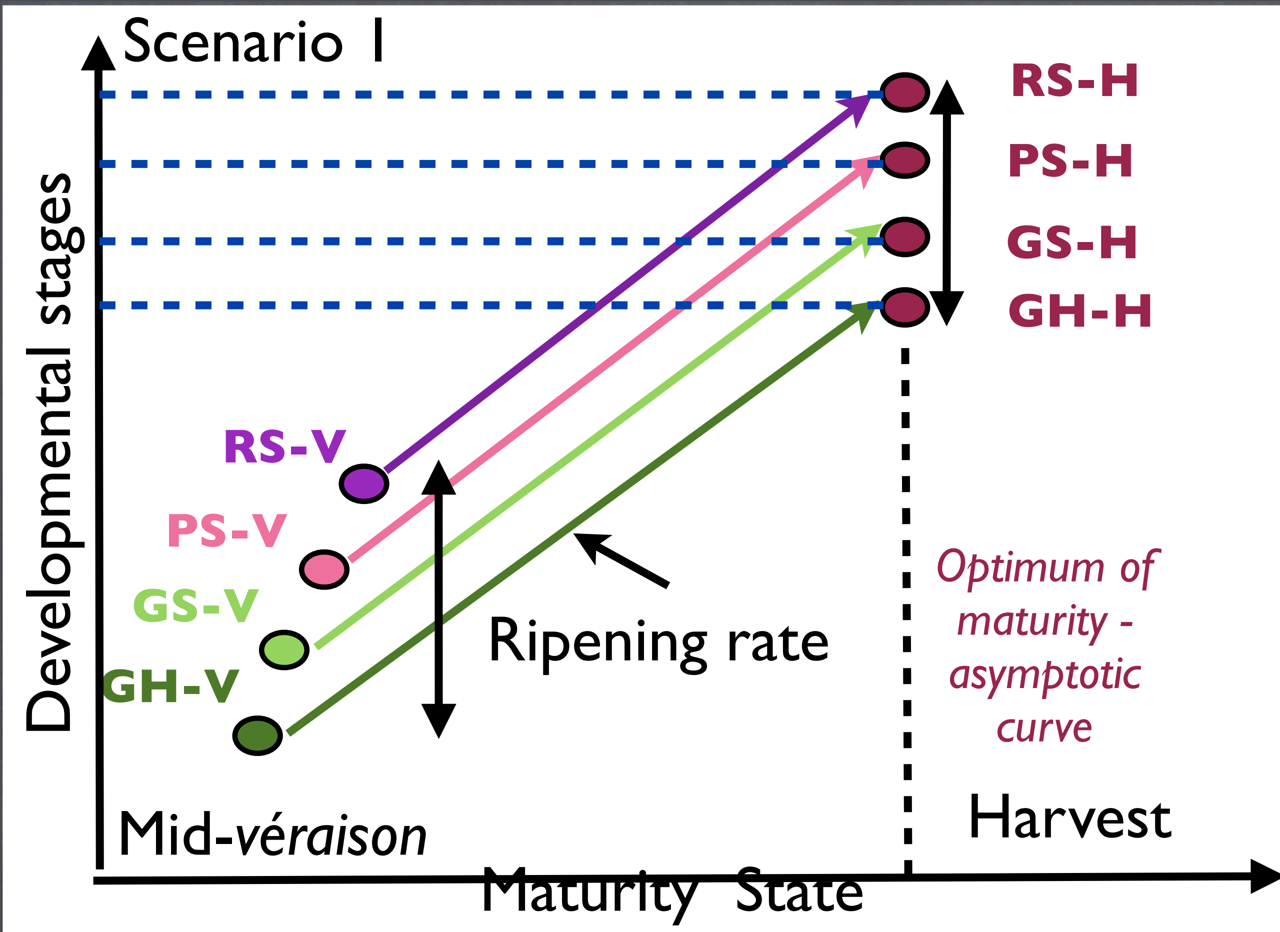

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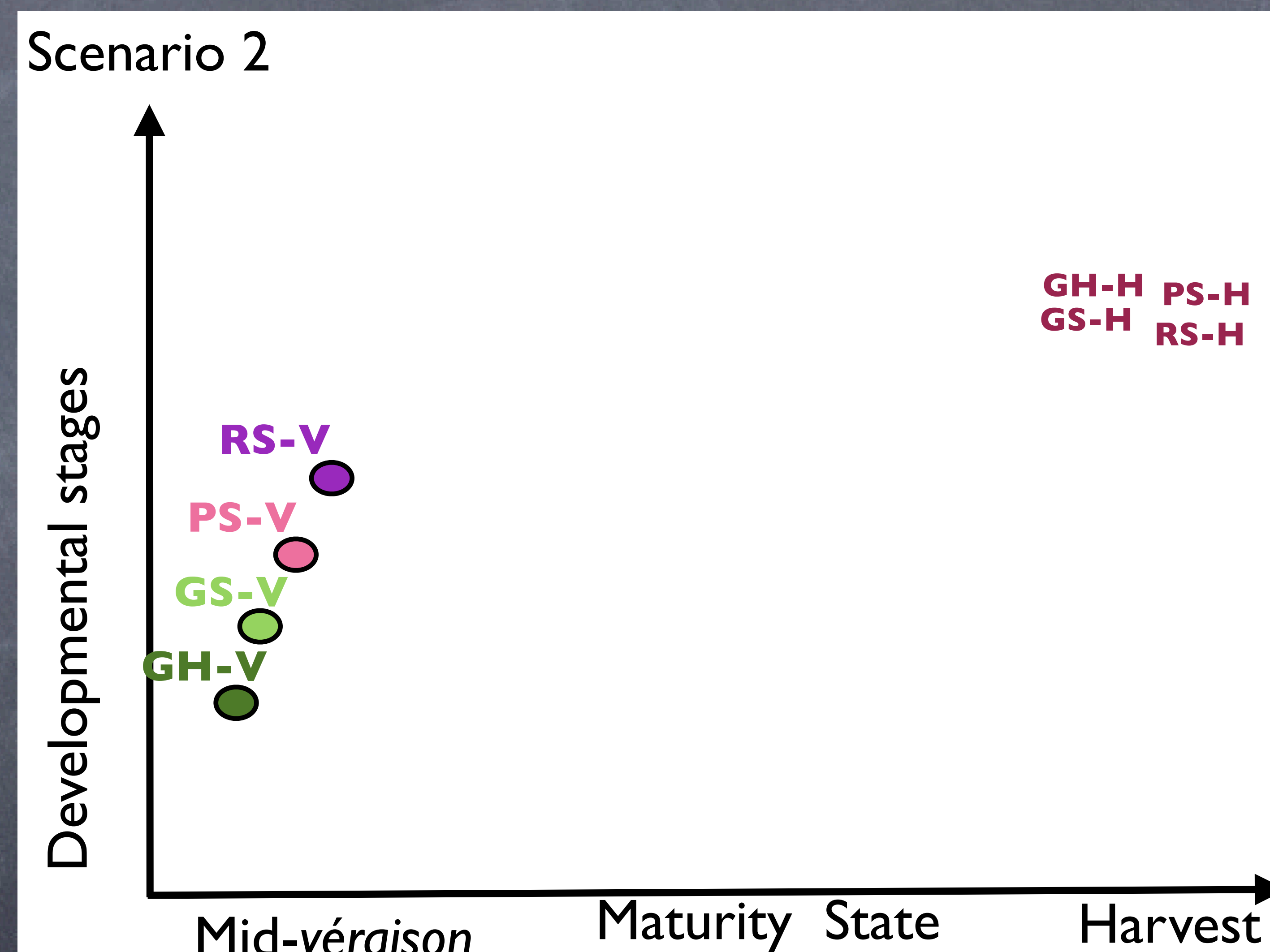

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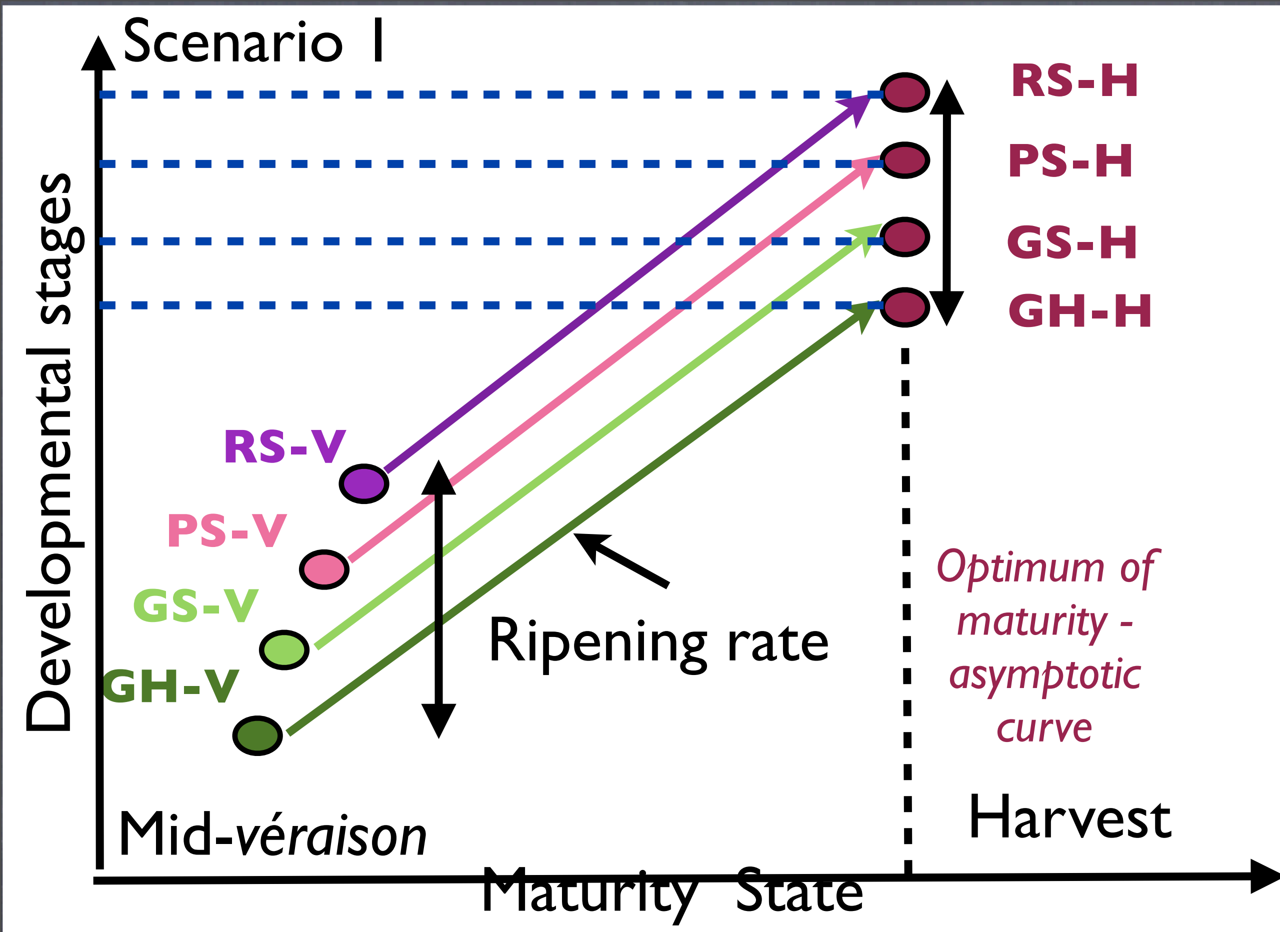

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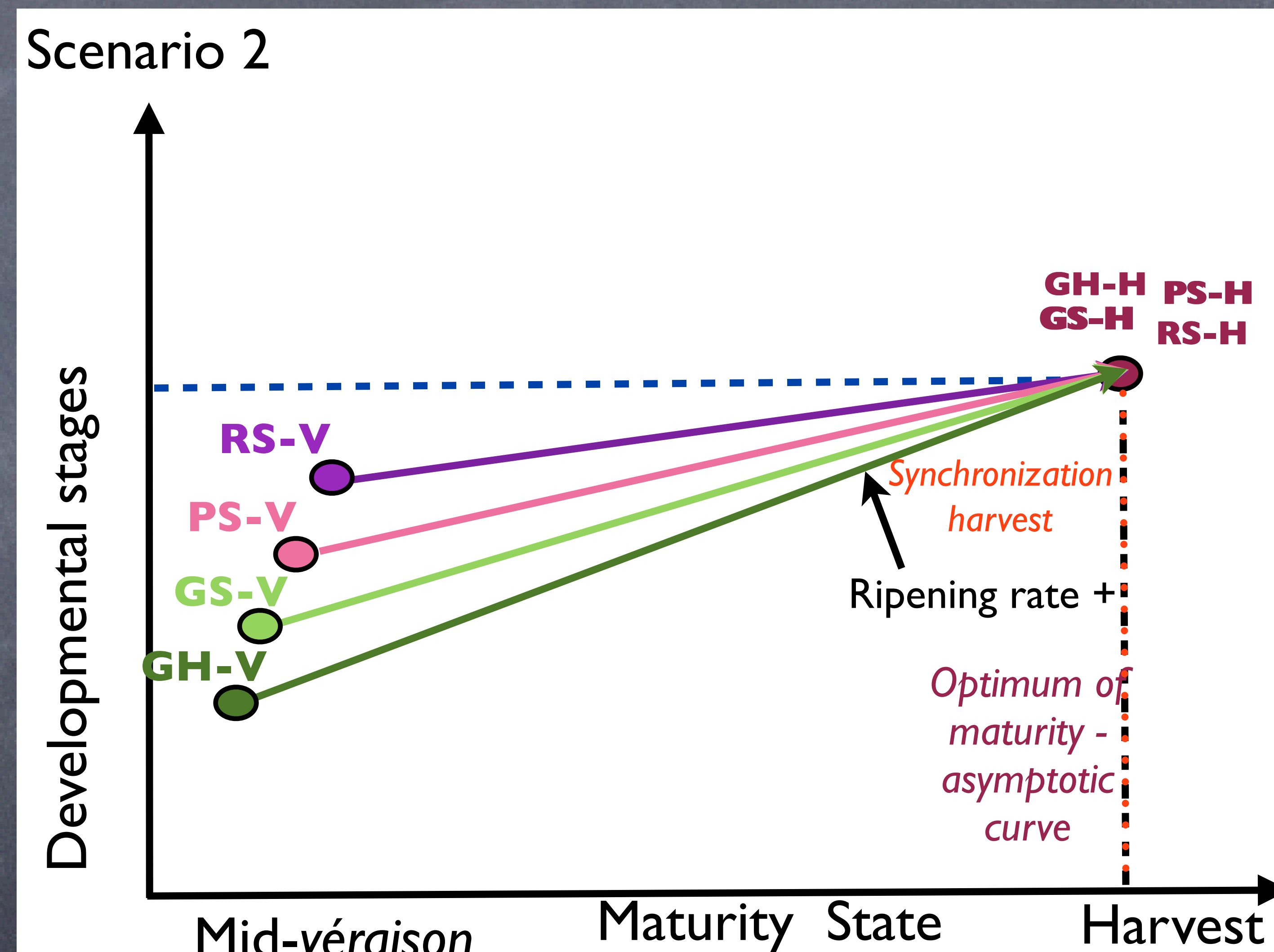

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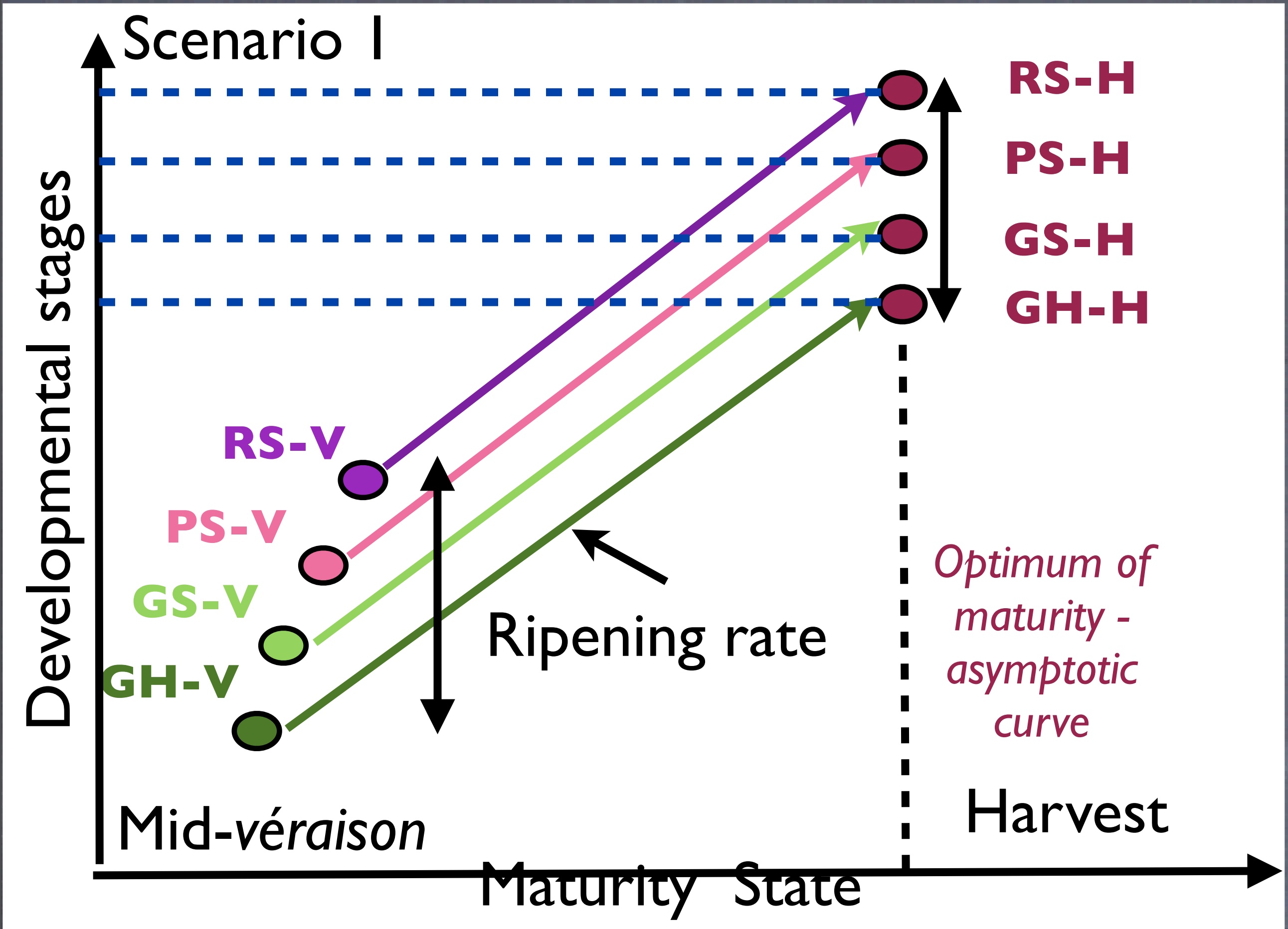

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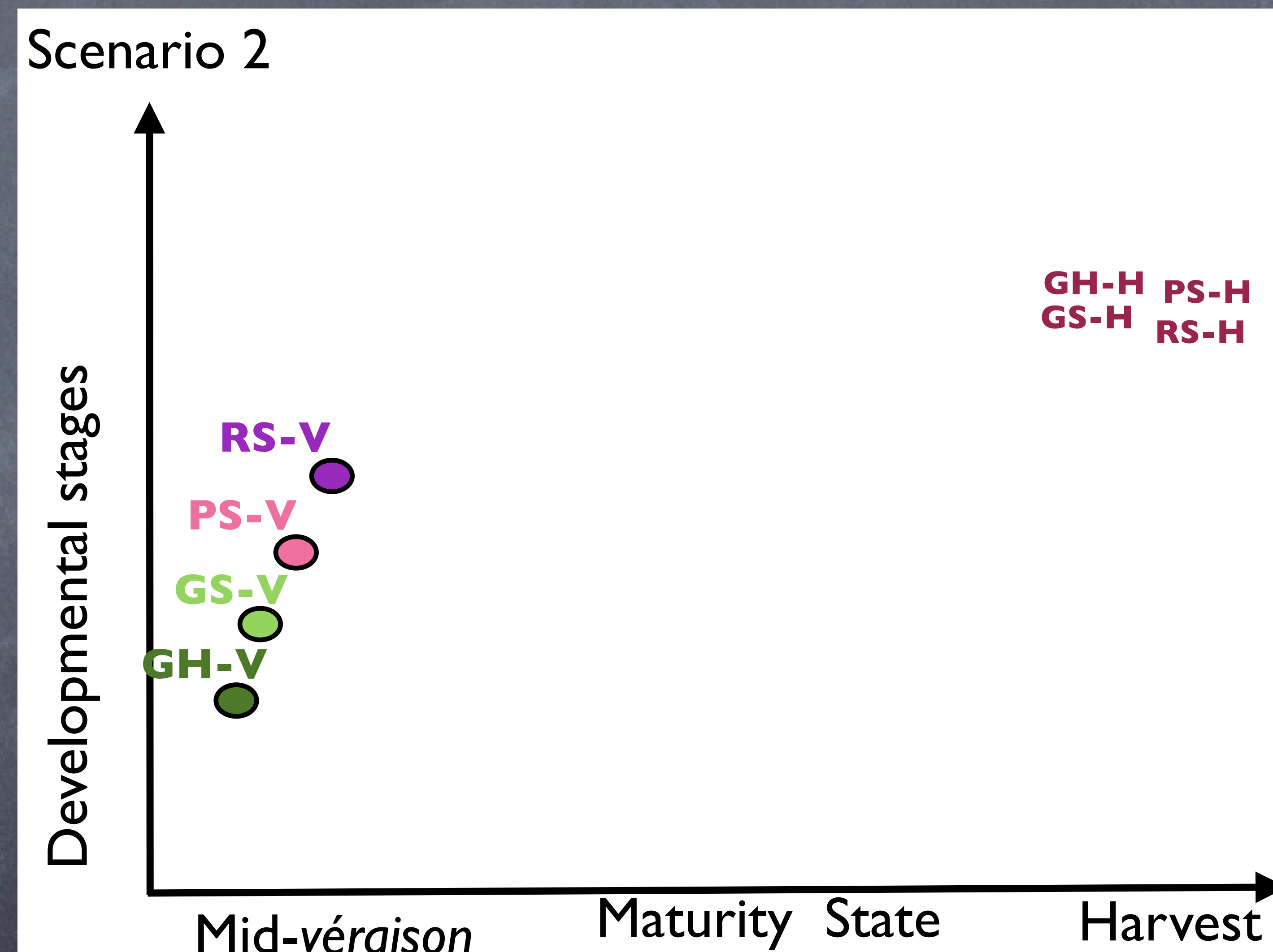

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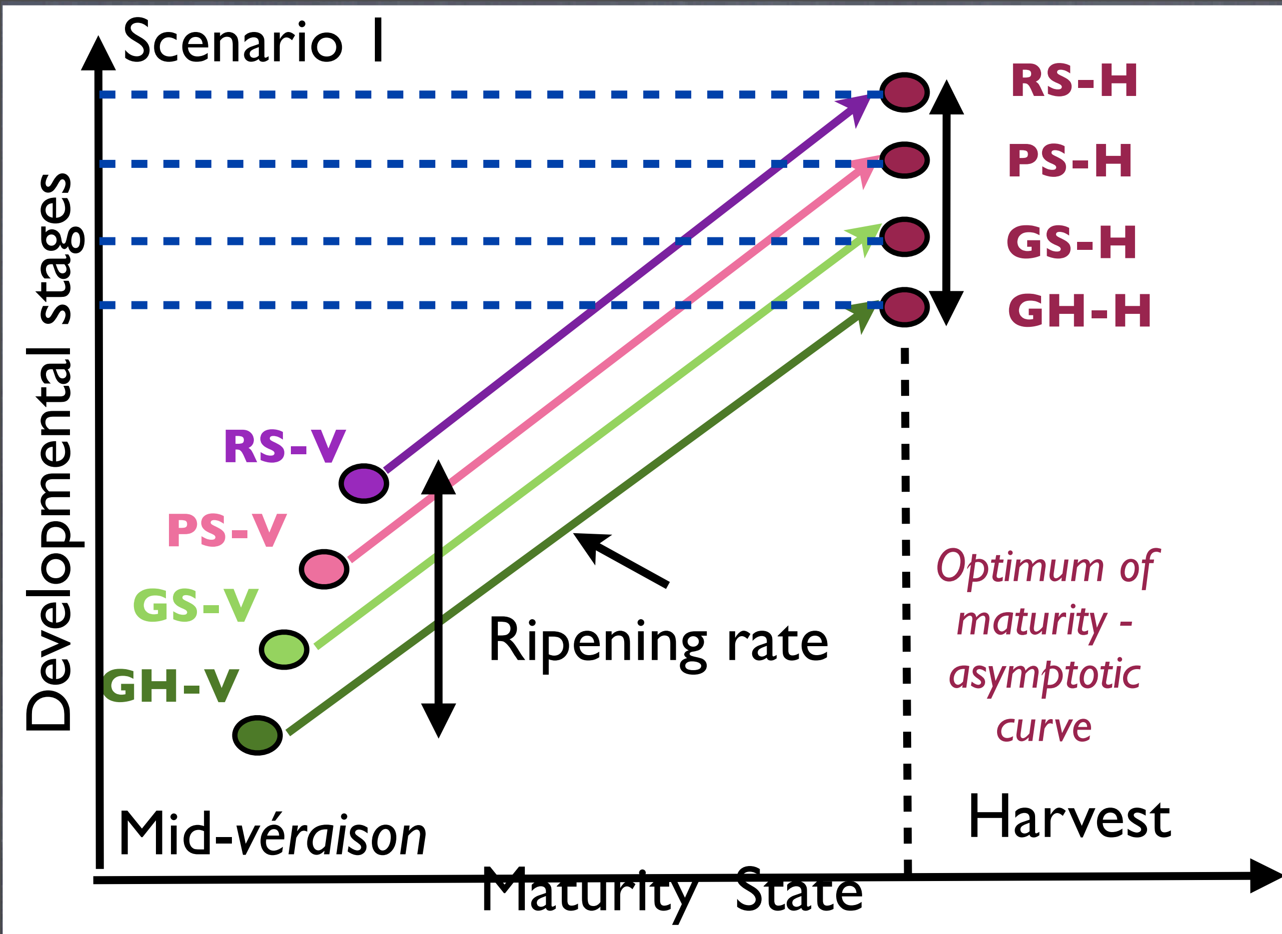

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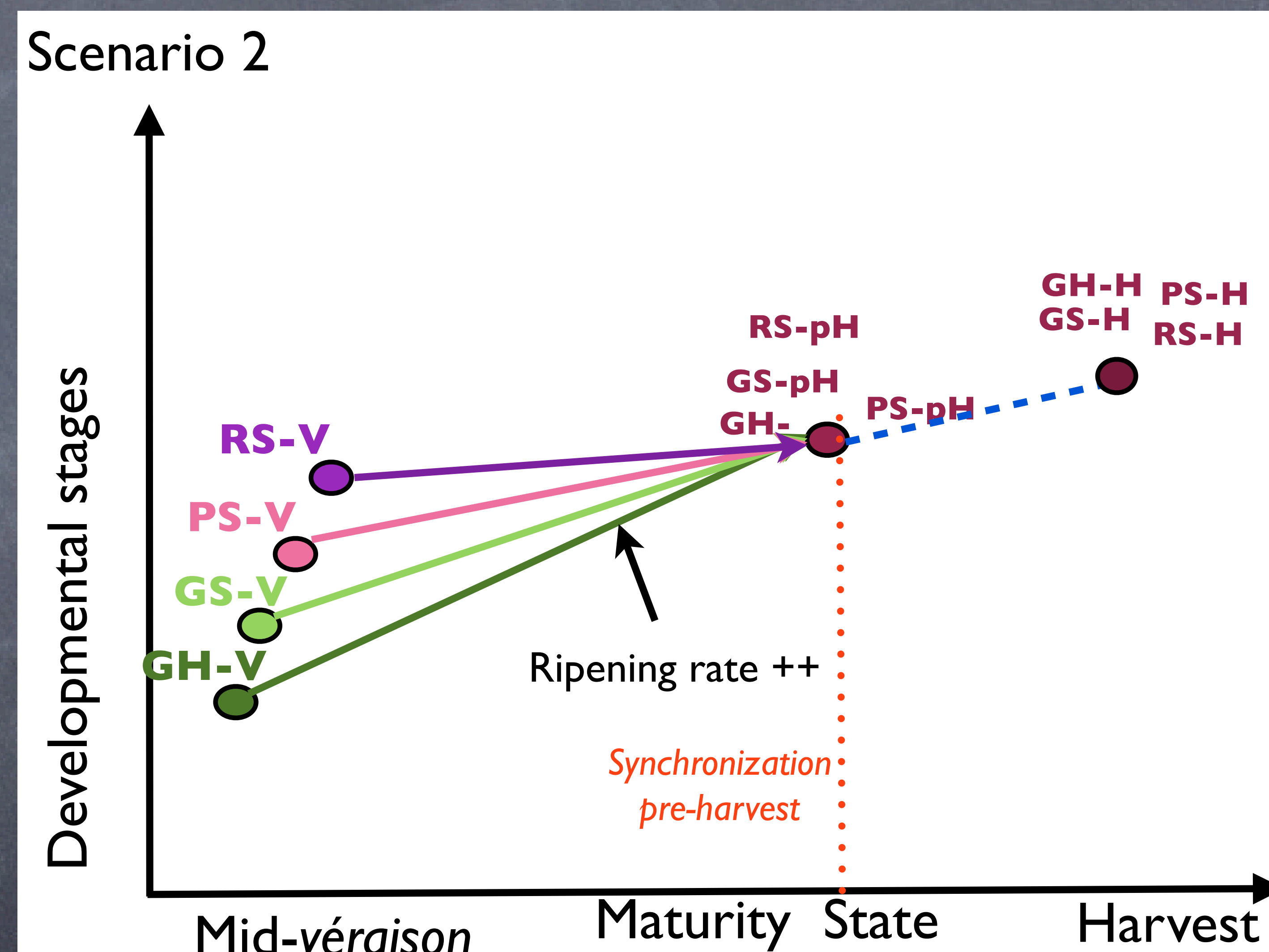

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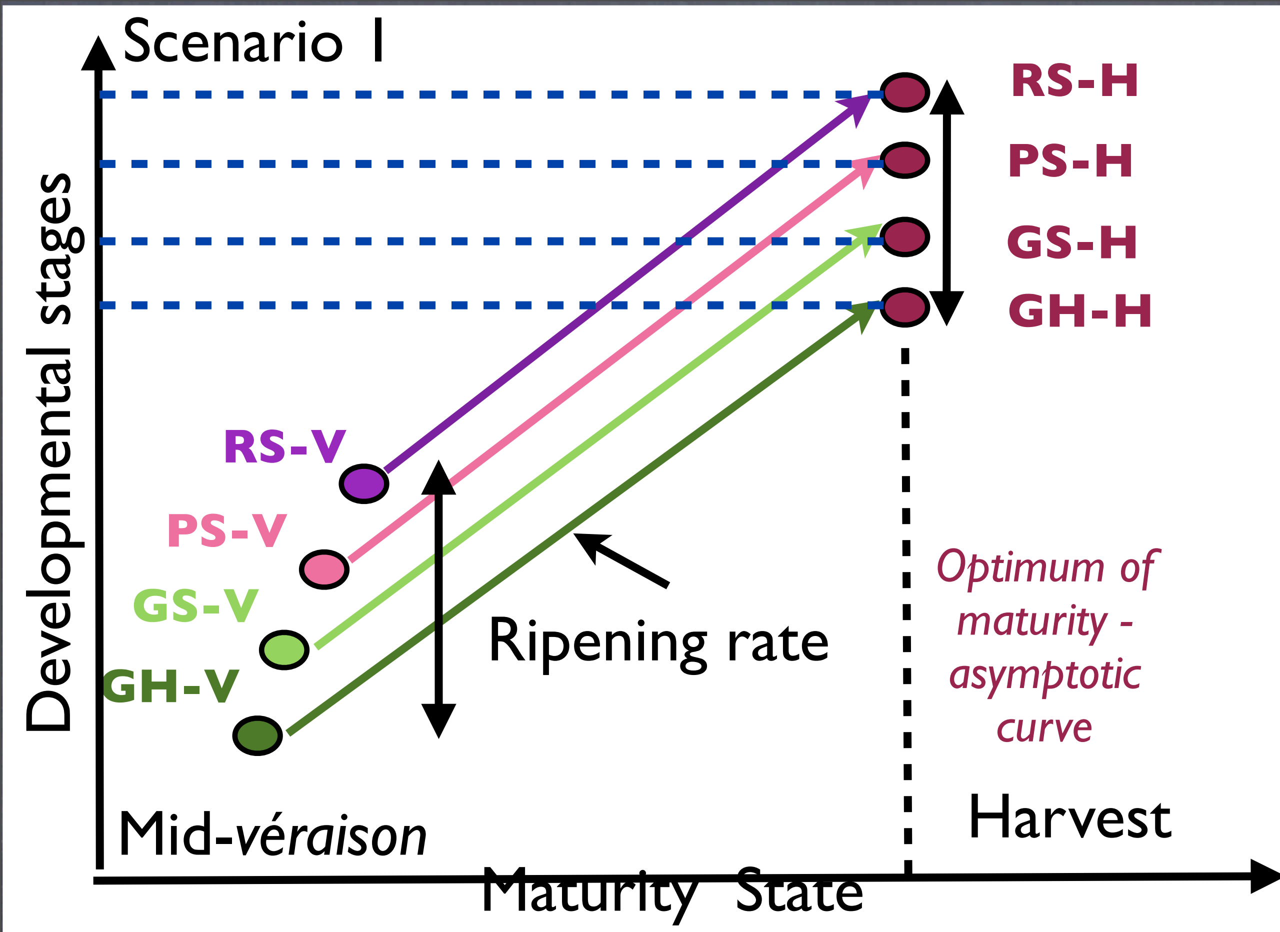

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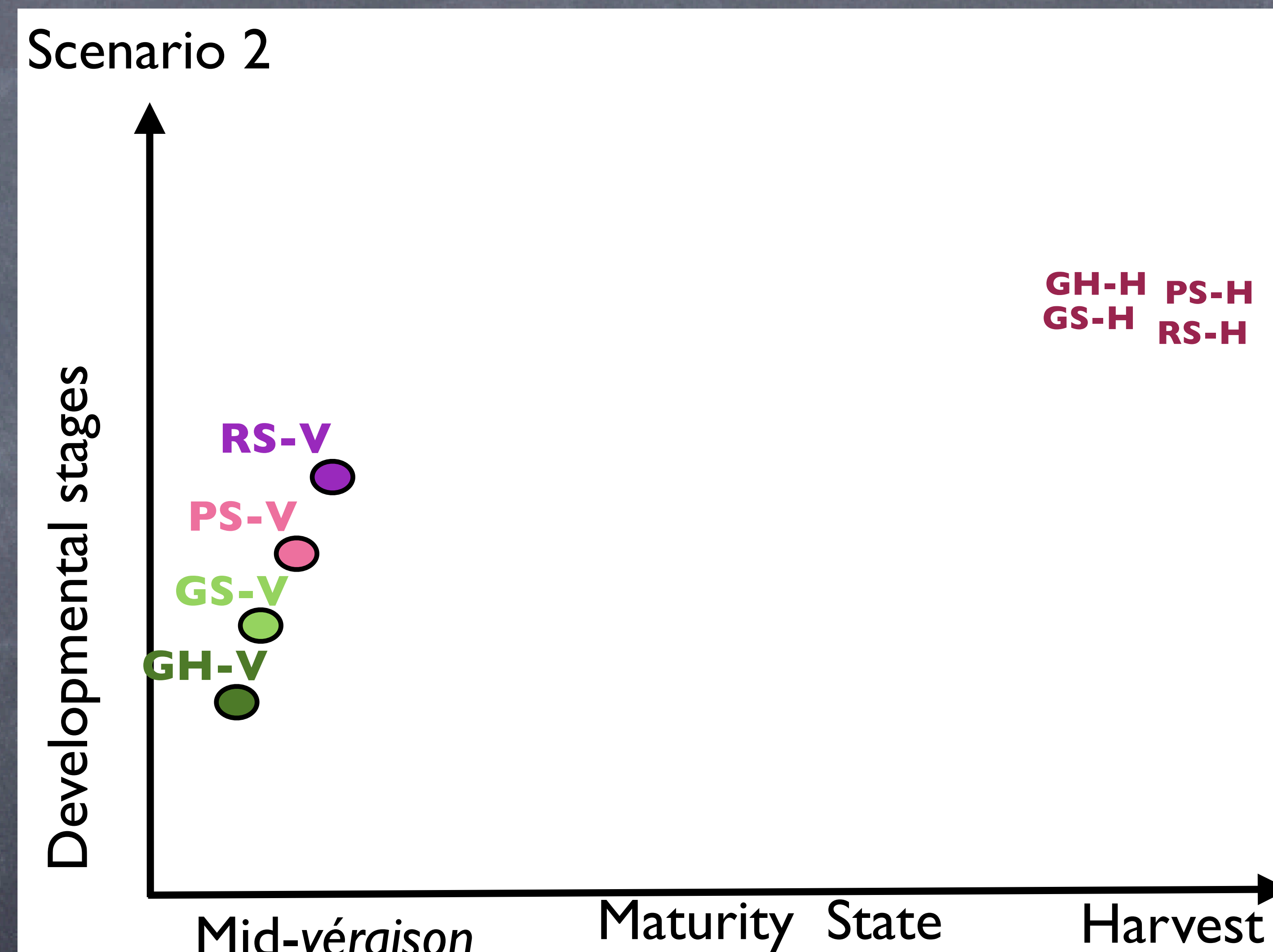

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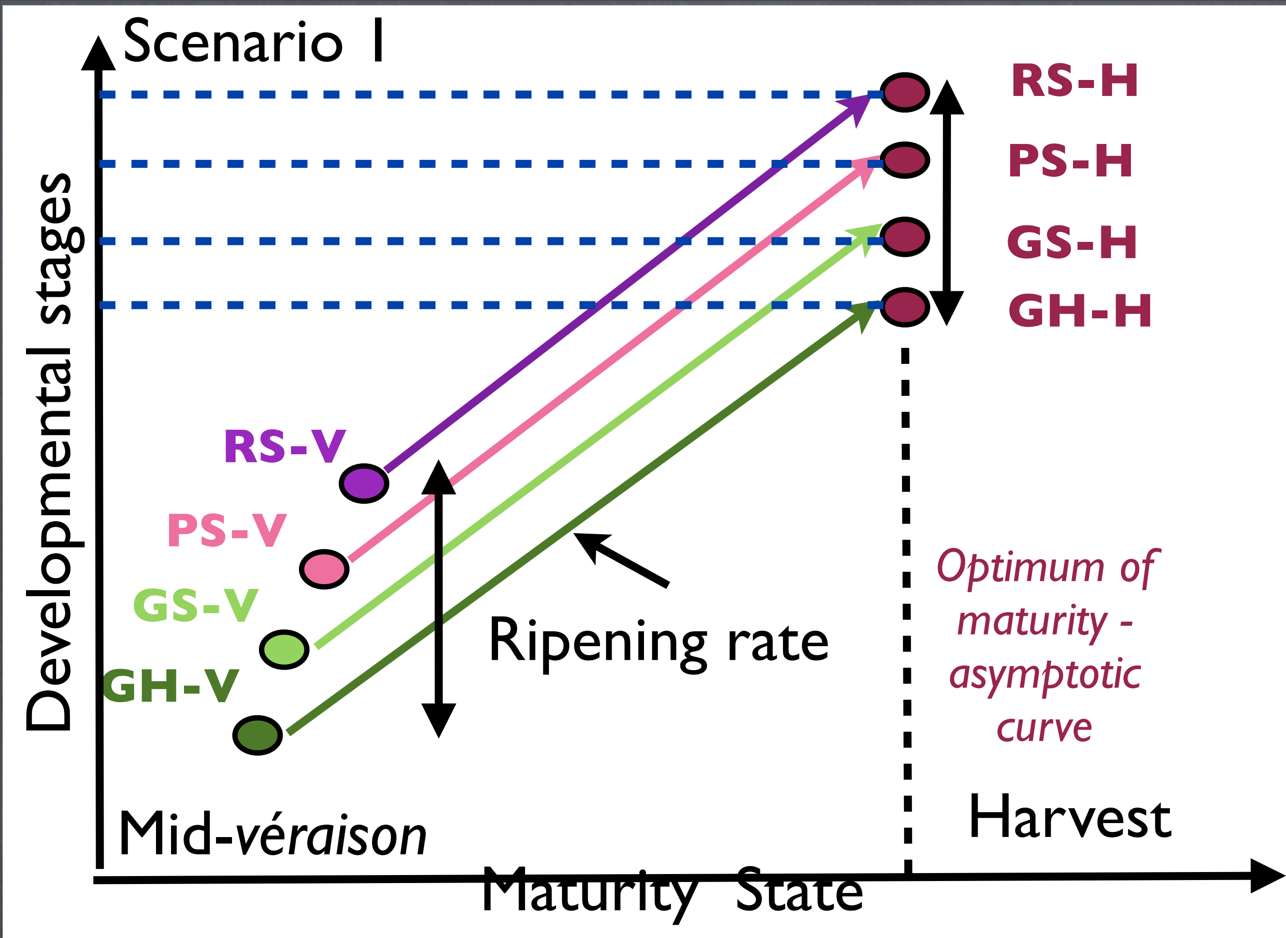

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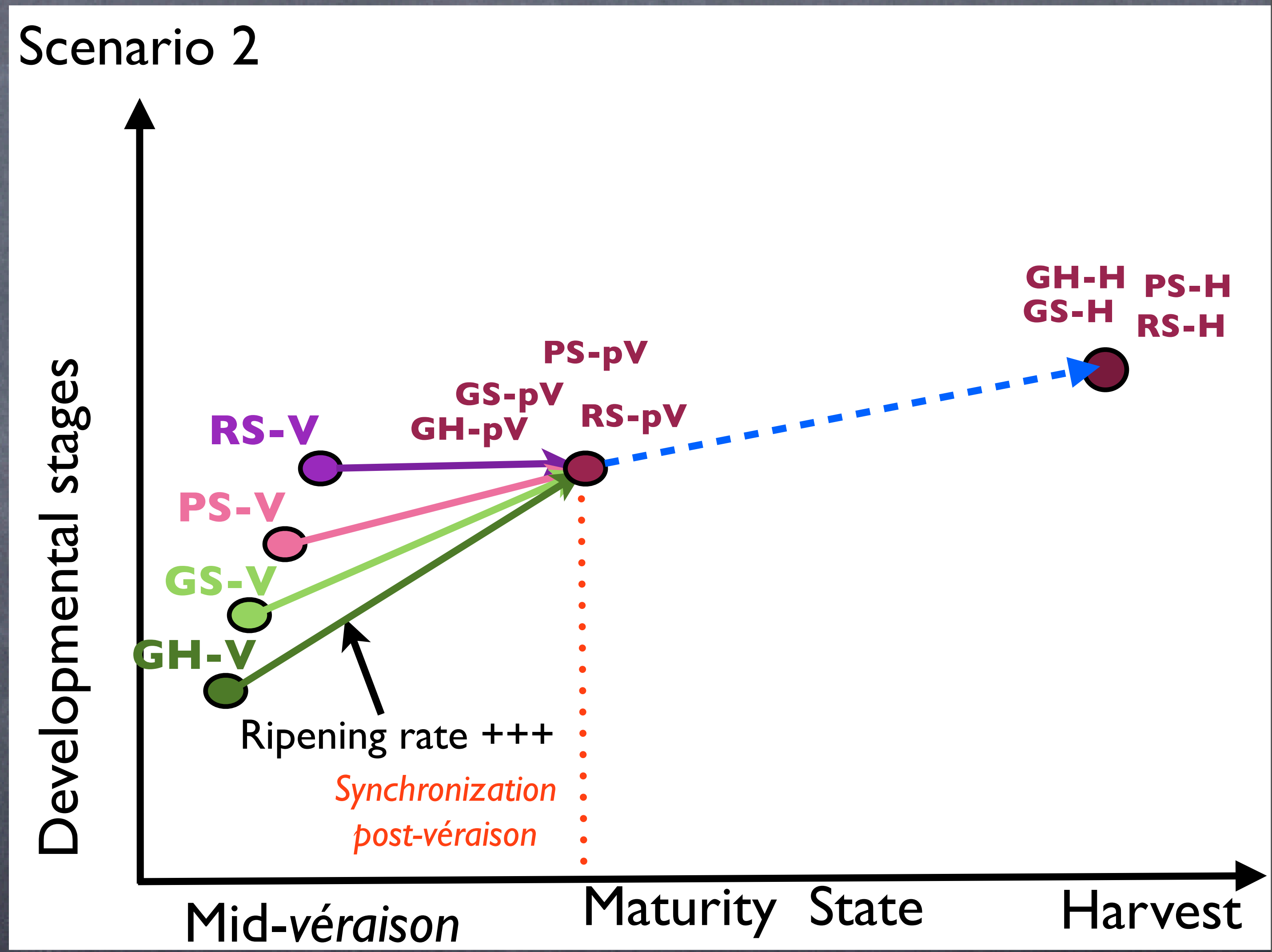

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Oregon Wine Research Institute
Oregon State University

Questions

Is there a reduction of berry developmental variability at harvest in Pinot Noir?

What is the contribution of each berry class to the overall fruit quality?

What is the mechanism ?

Appropriate way to correlate berry classes and a berry developmental stage?

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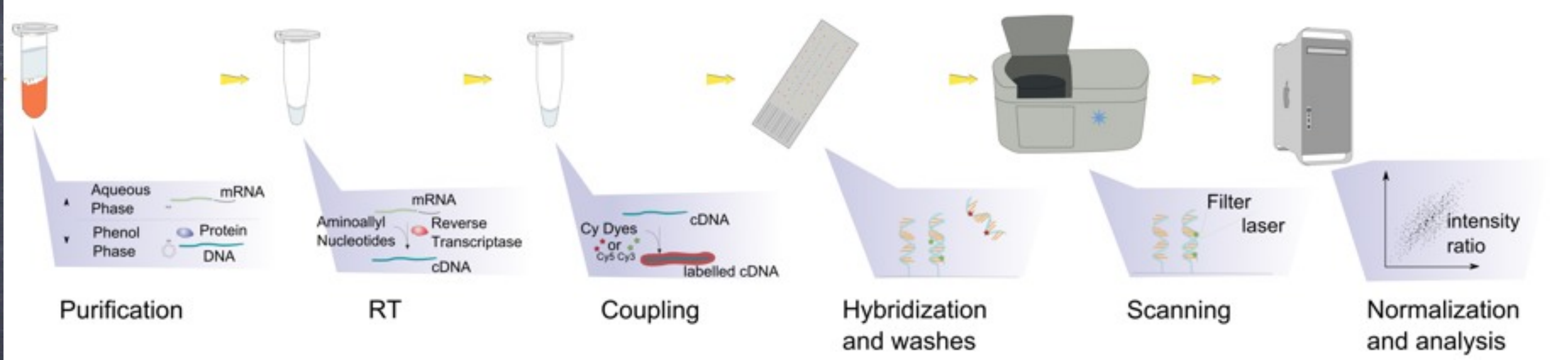
How describe a developmental stage?

Using genomic hybridization or sequencing technology as fingerprint to describe a given developmental stage or a tissue

DNA microarray experiment



Generate the most comprehensive snapshot at the gene expression level to describe a particular stage

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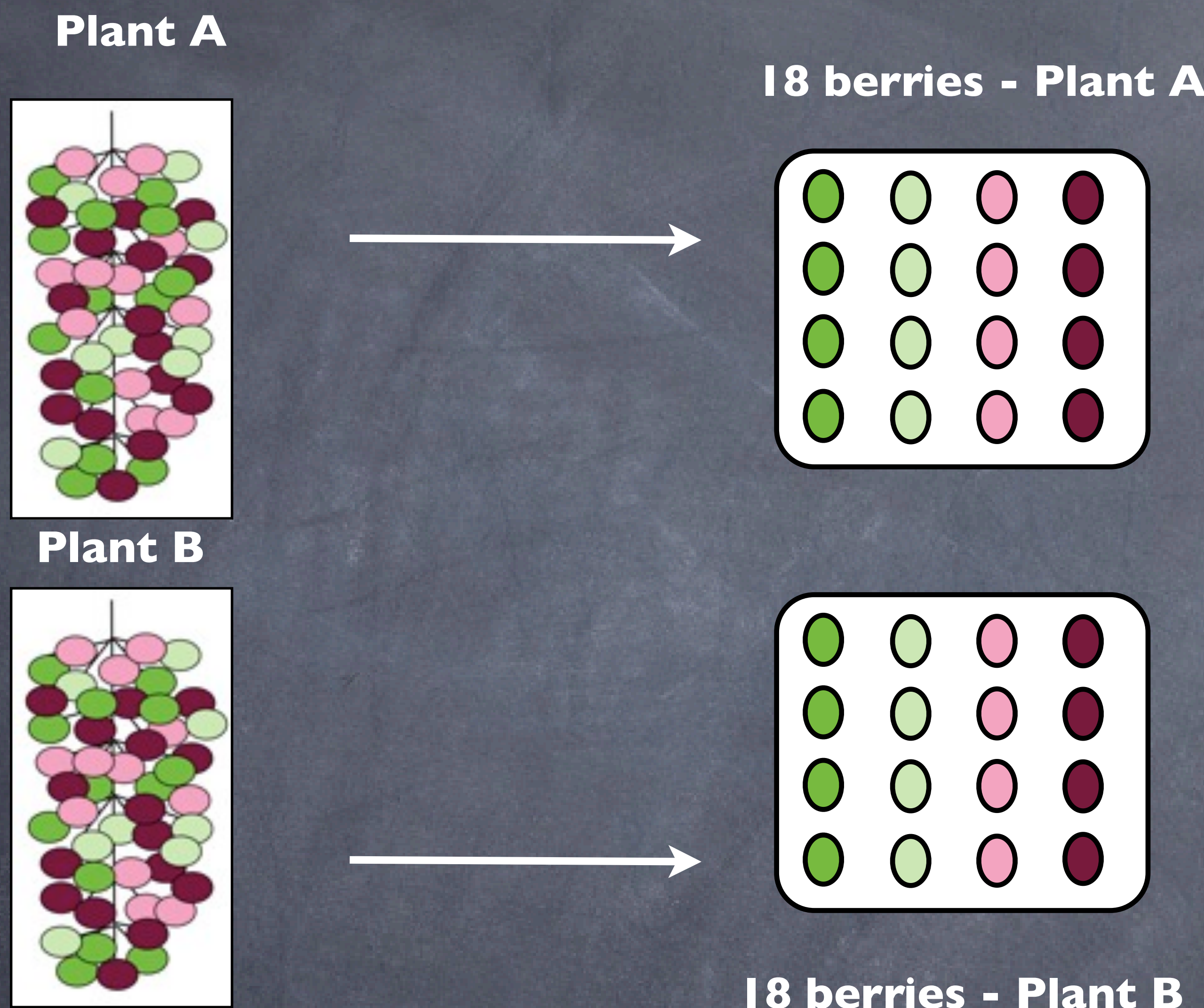
A global gene expression profile reflects a developmental stage (Lund et al., 2008)



Berries from same class have similar global transcript profile signatures

Significance test (global transcript profile versus phenotypic variables)

Phenotypic variable	Significance ($p<0.05$)
Plant	0.72
Position in the cluster	0.54
Diameter	0.18
Weight	0.10
Soluble Solids	0.04
Color + Firmness	0.08
Firmness	0.07
Color	0.03

Global transcript signatures are significantly correlated to some phenotypic variables

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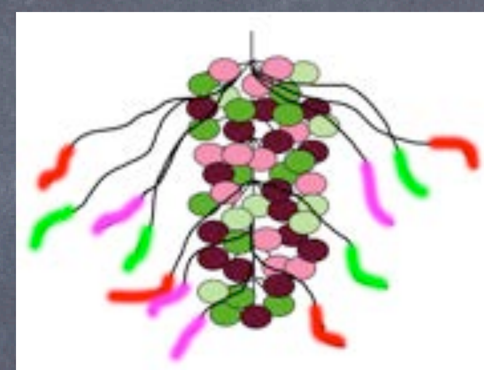
Phenological measurements of individual berries at mid-véraison and harvest

Mid-véraison | *Harvest*

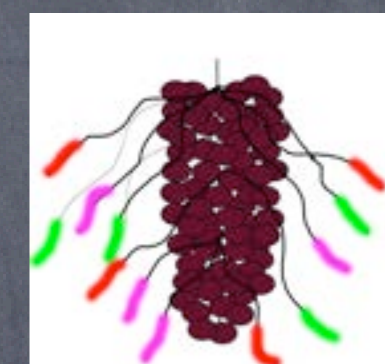
Berry Formation | PV | Véraison | Berry Ripening

GH-V *GS-V* *PS-V* *RS-V*

GH-H *GS-H* *PS-H* *RS-H*

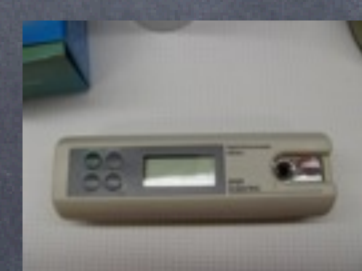


6 plants
5 clusters per plant
5 berries per class
Four berry classes

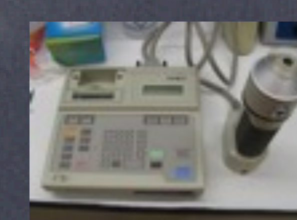


Monitoring individual berries from *véraison* to harvest

Refractometer



Chromameter (lightness, hue, chroma)

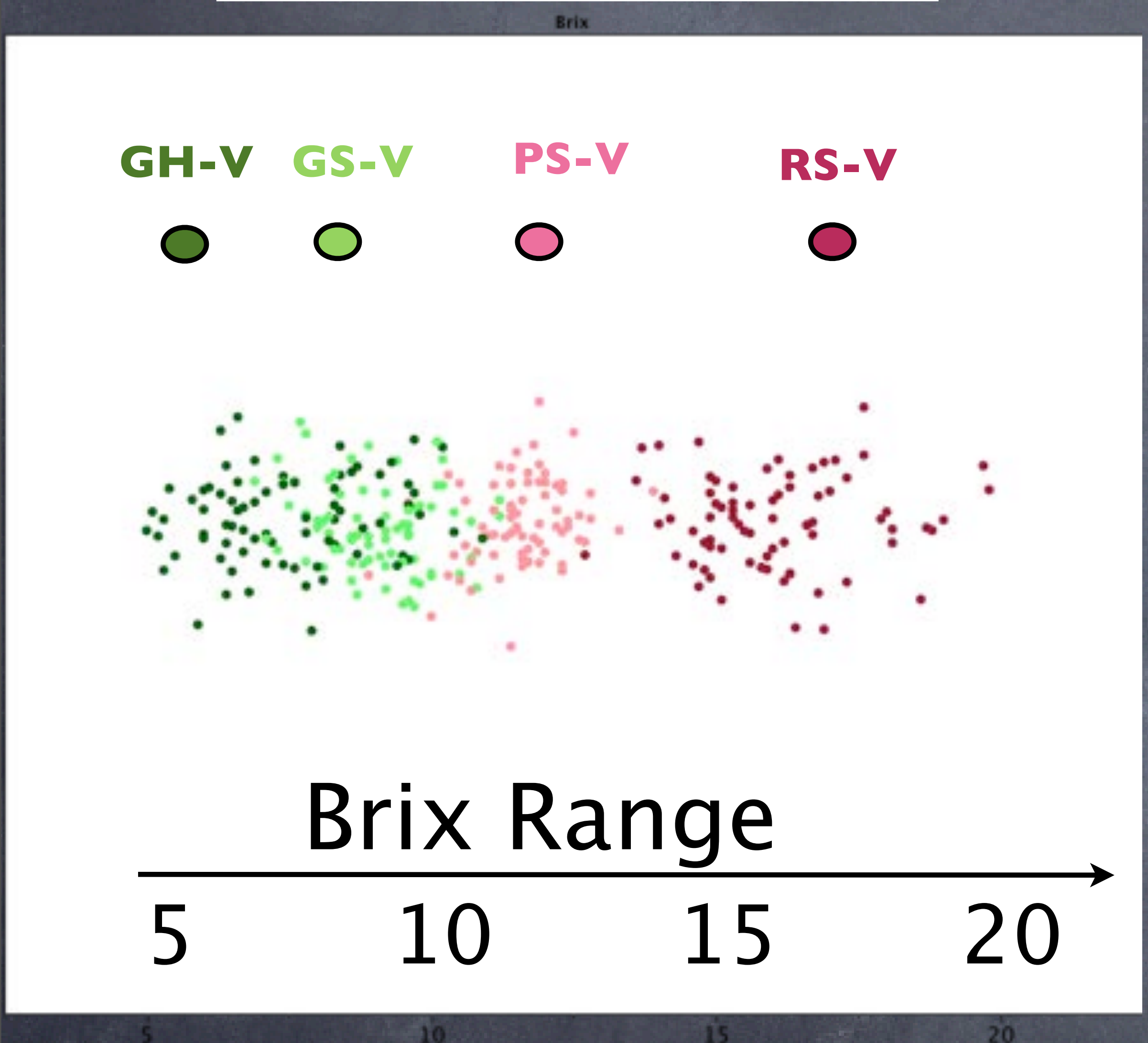


Multivariate Analysis of Variance, Principle Component Analysis, Discriminant Analysis

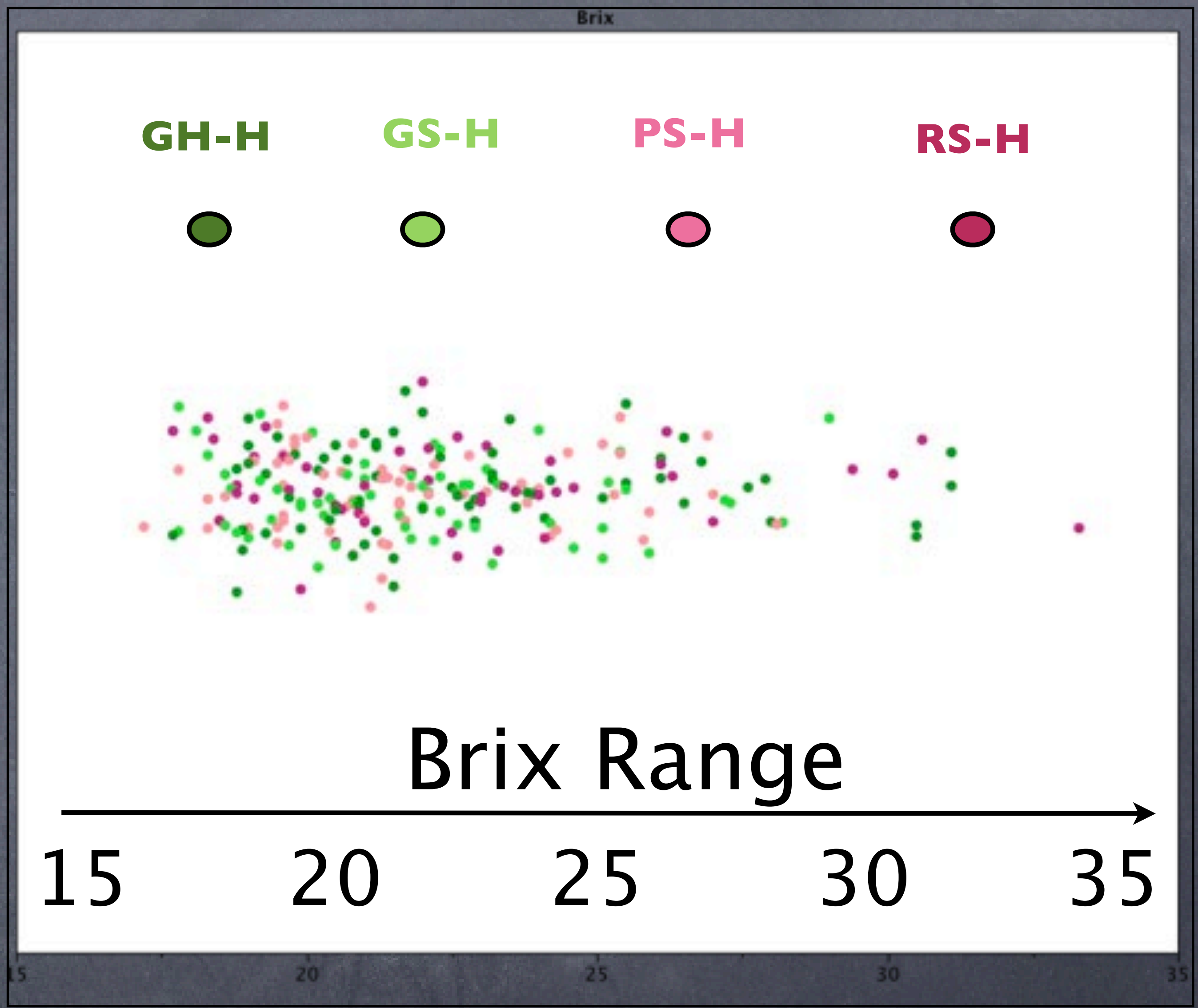
Laurent Deluc
Oregon Wine Research Institute
OSU Oregon State University

Brix values of individual at mid-véraison and harvest

Mid-véraison:



Harvest:



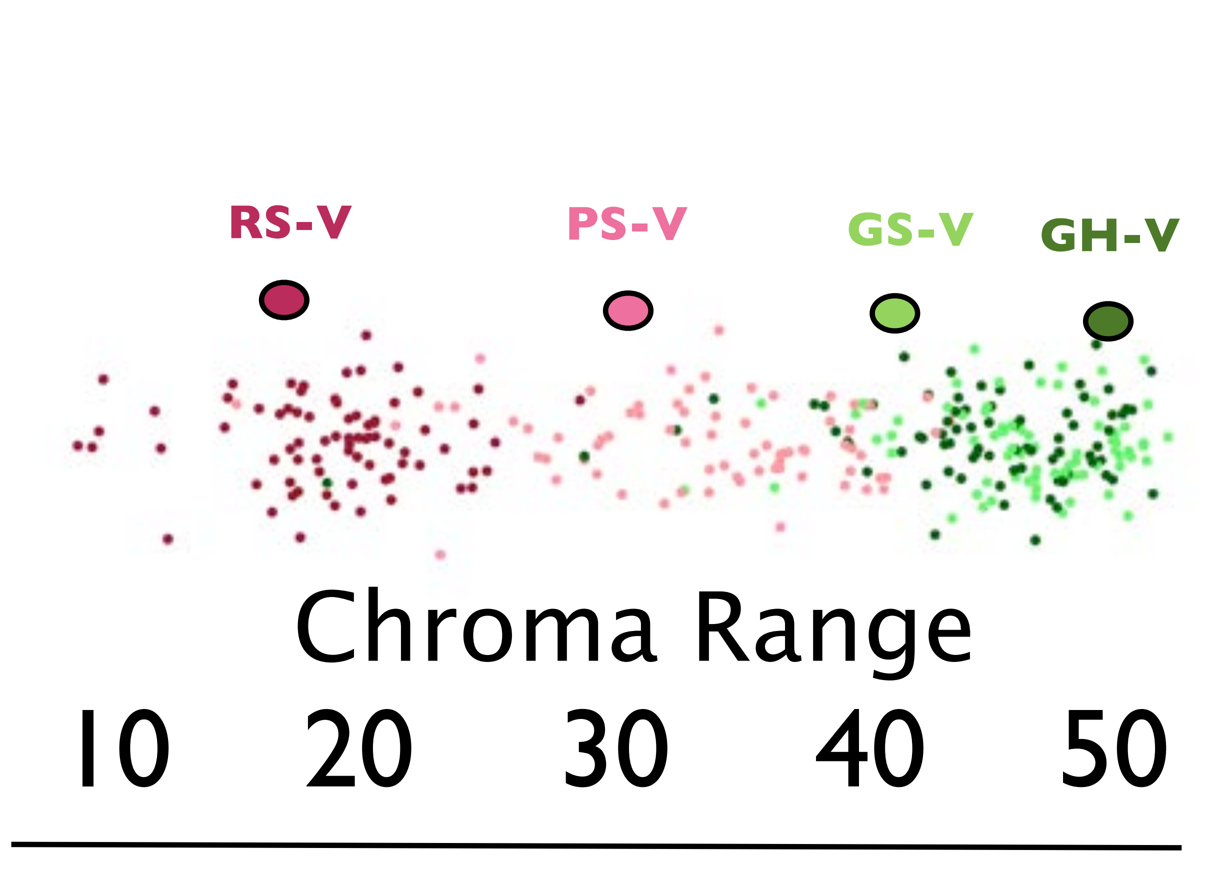
Laurent Deluc

Oregon Wine Research Institute

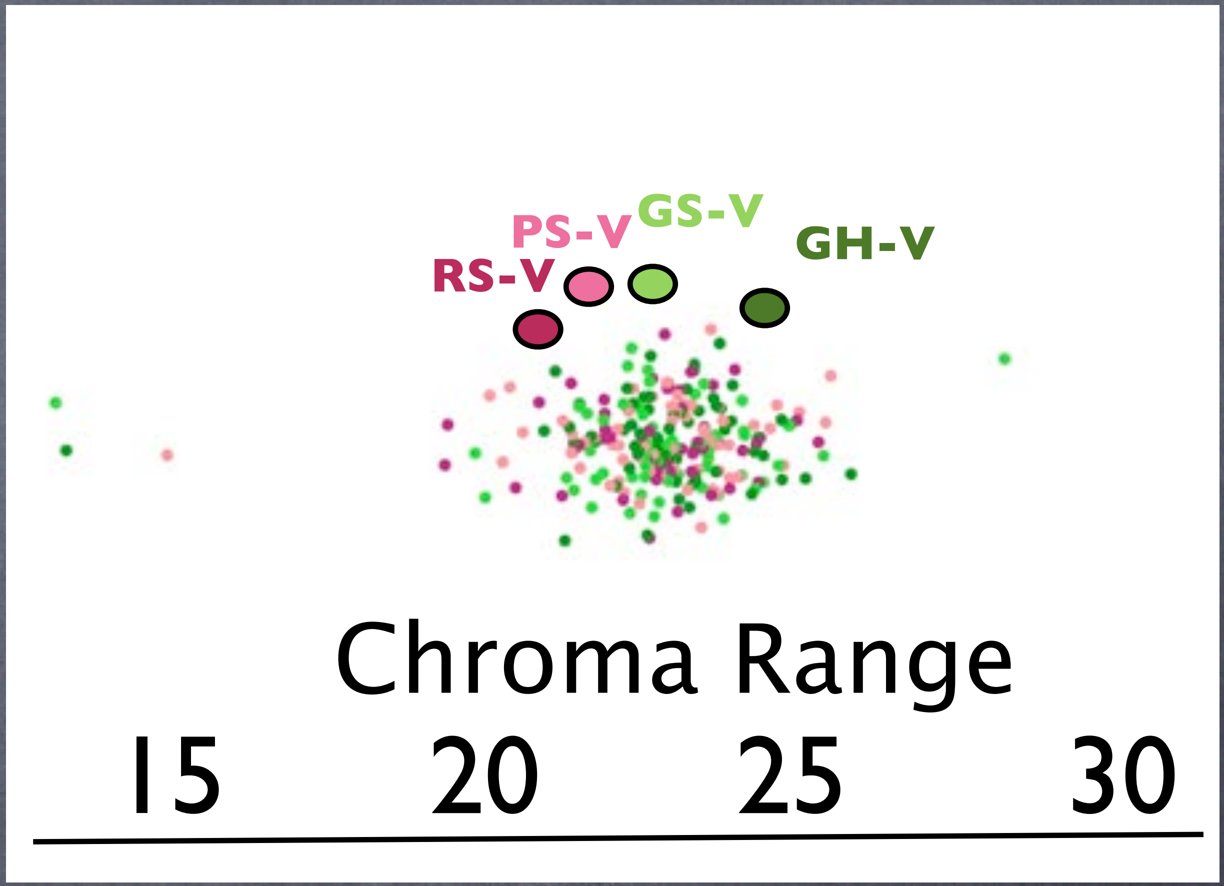
Oregon State University

Chroma values of individual at mid-véraison and harvest

Mid-véraison:



Harvest:



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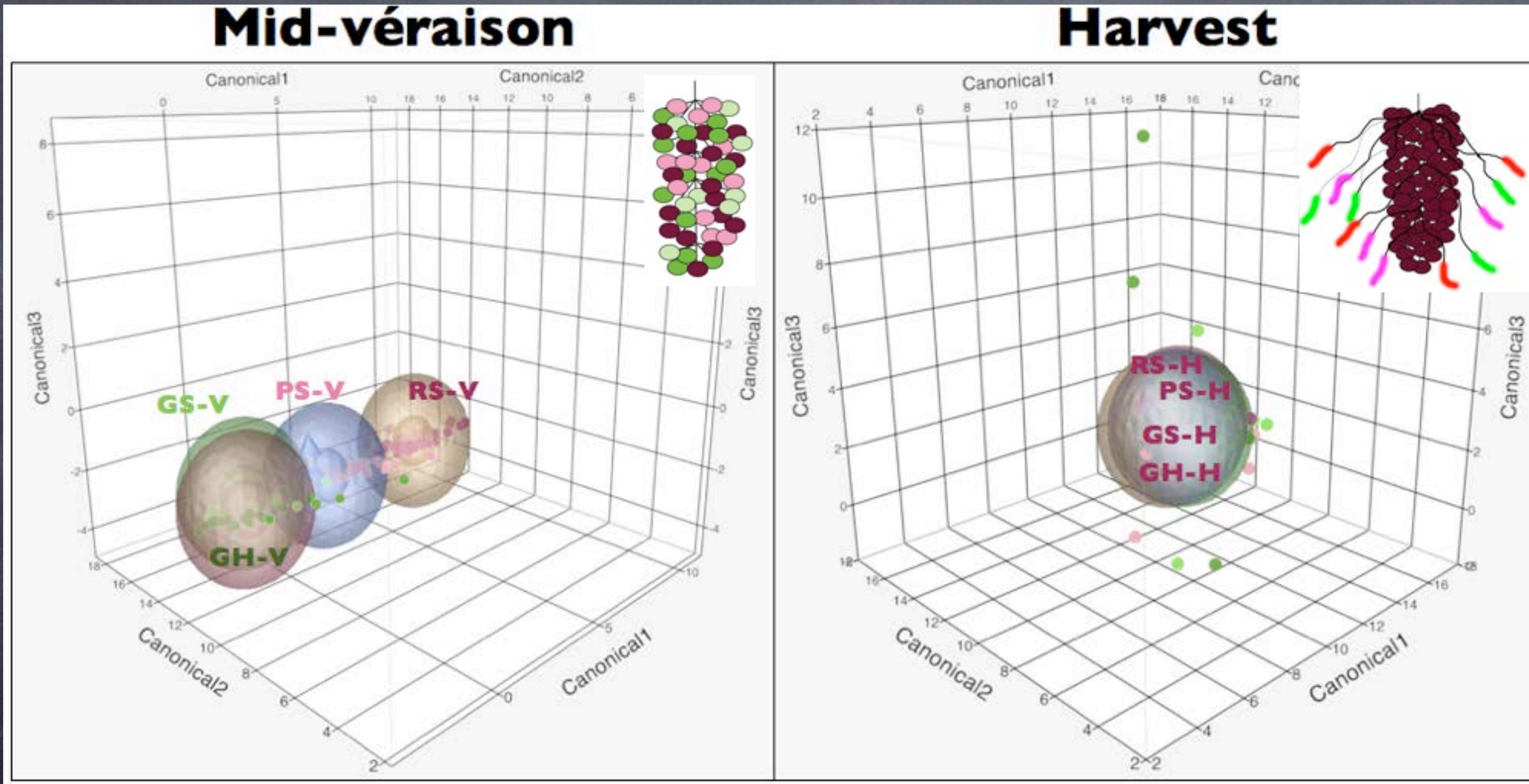
Oregon Wine Research Institute

OSU Oregon State University

Discriminant Analyses using Brix, lightness, hue and chroma



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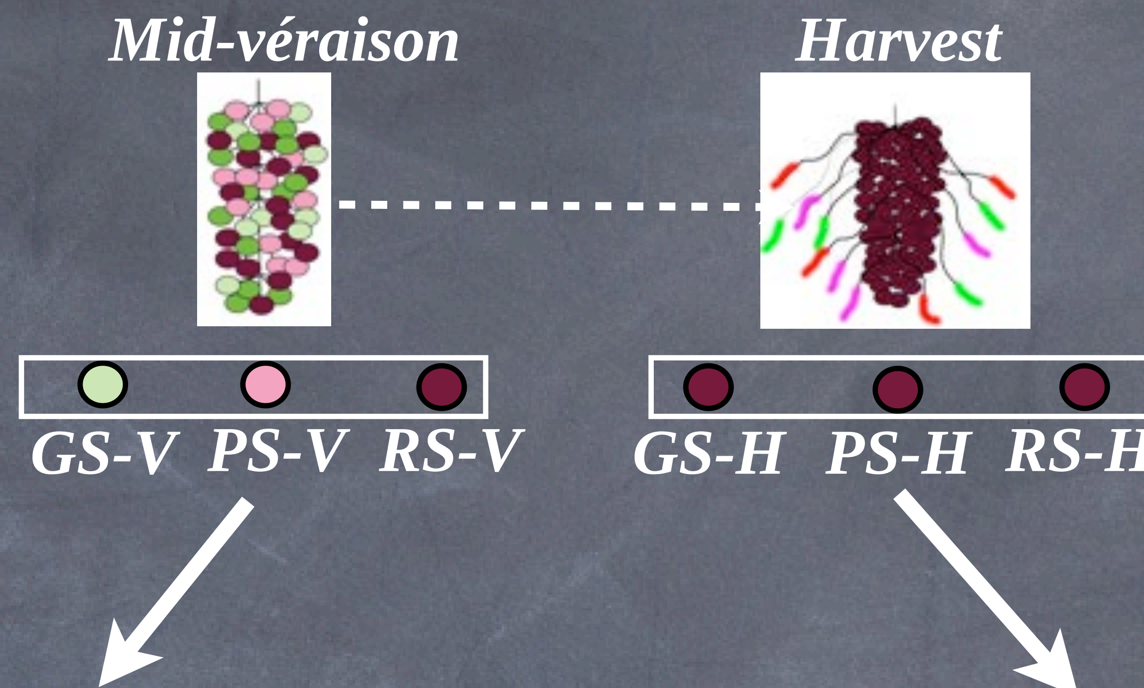
Oregon Wine Research Institute

Oregon State University

Transcriptomic experiment (genome hybridization)



27 arrays (3 tissues, 3 plants, 3 berry classes)

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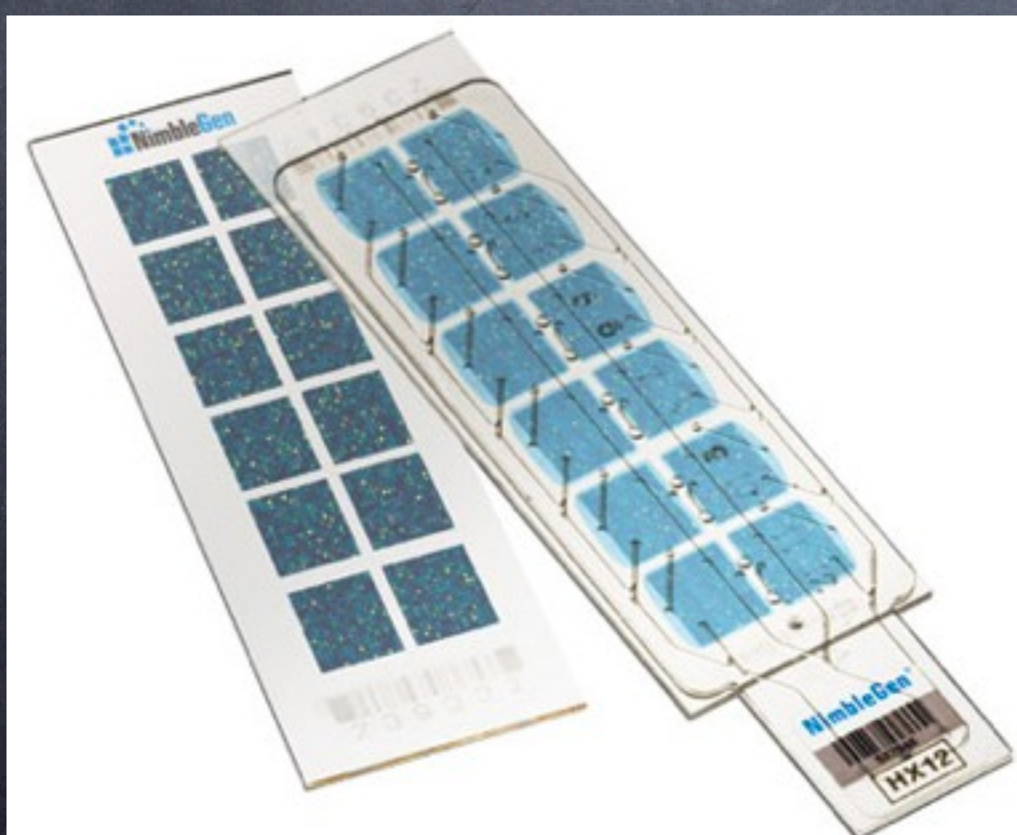
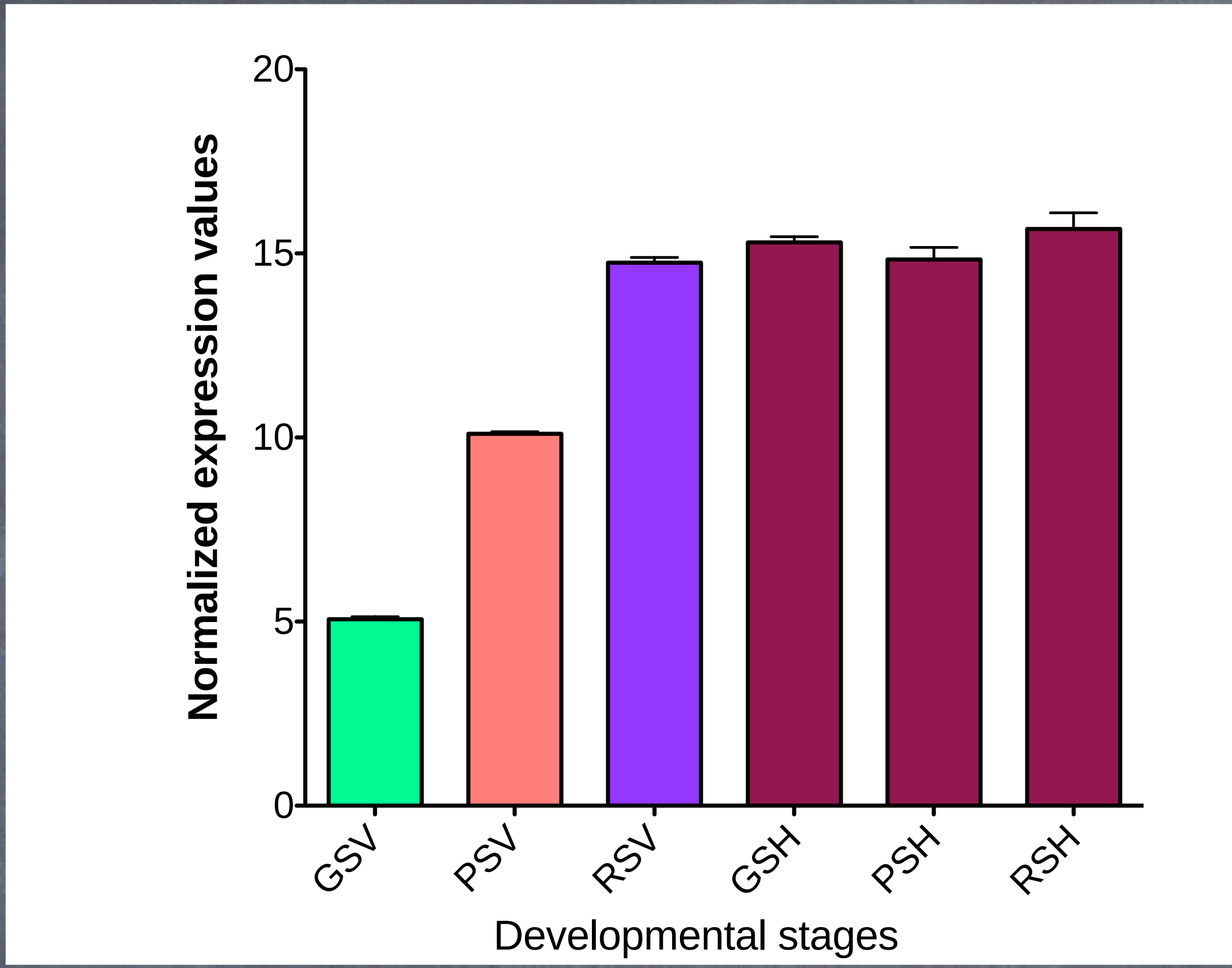


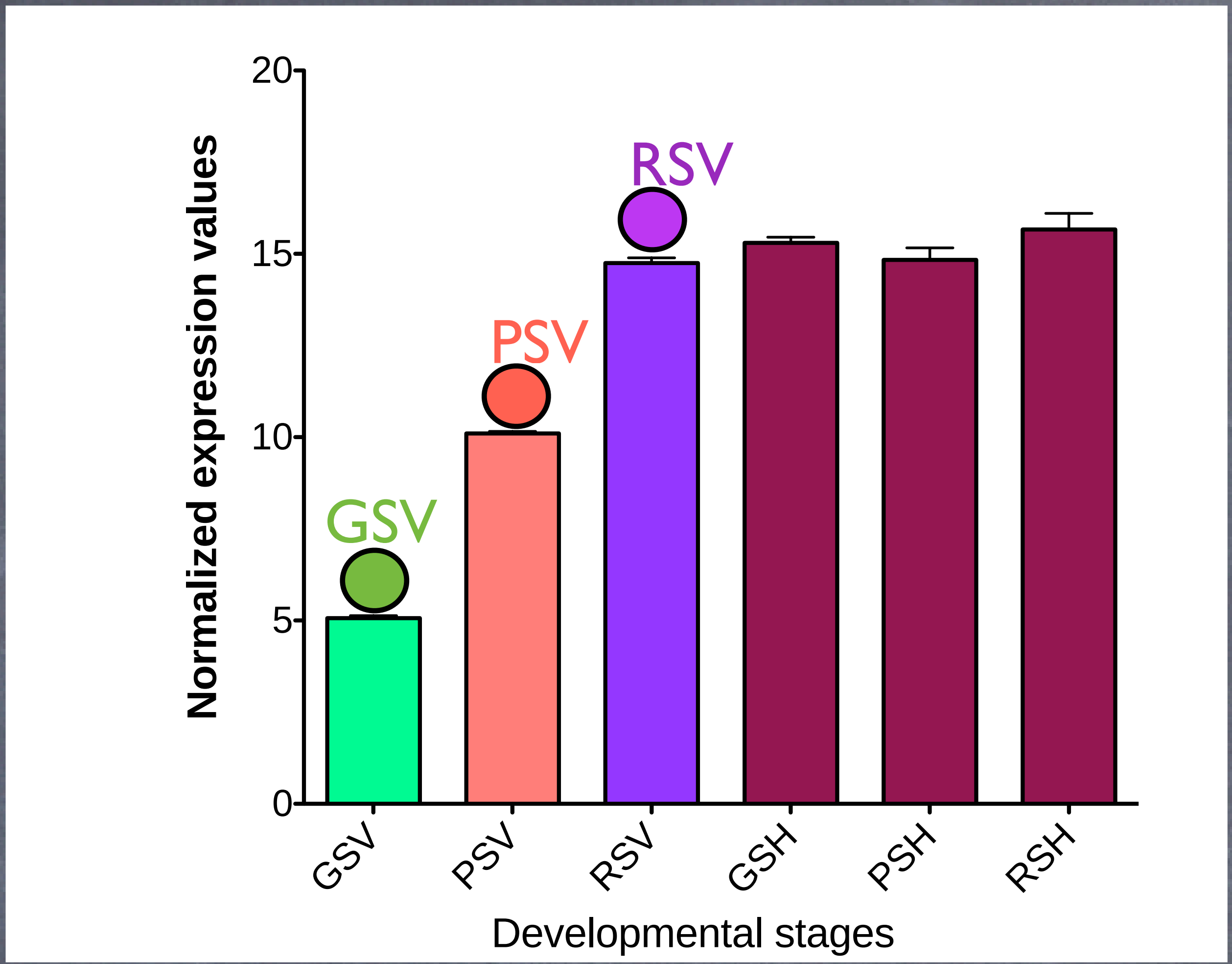
Nimblegen Grape whole transcriptome ~29,550 genes

Transcript profile of a UFGT gene



For 29,550 genes

Transcript profile of a UFGT gene



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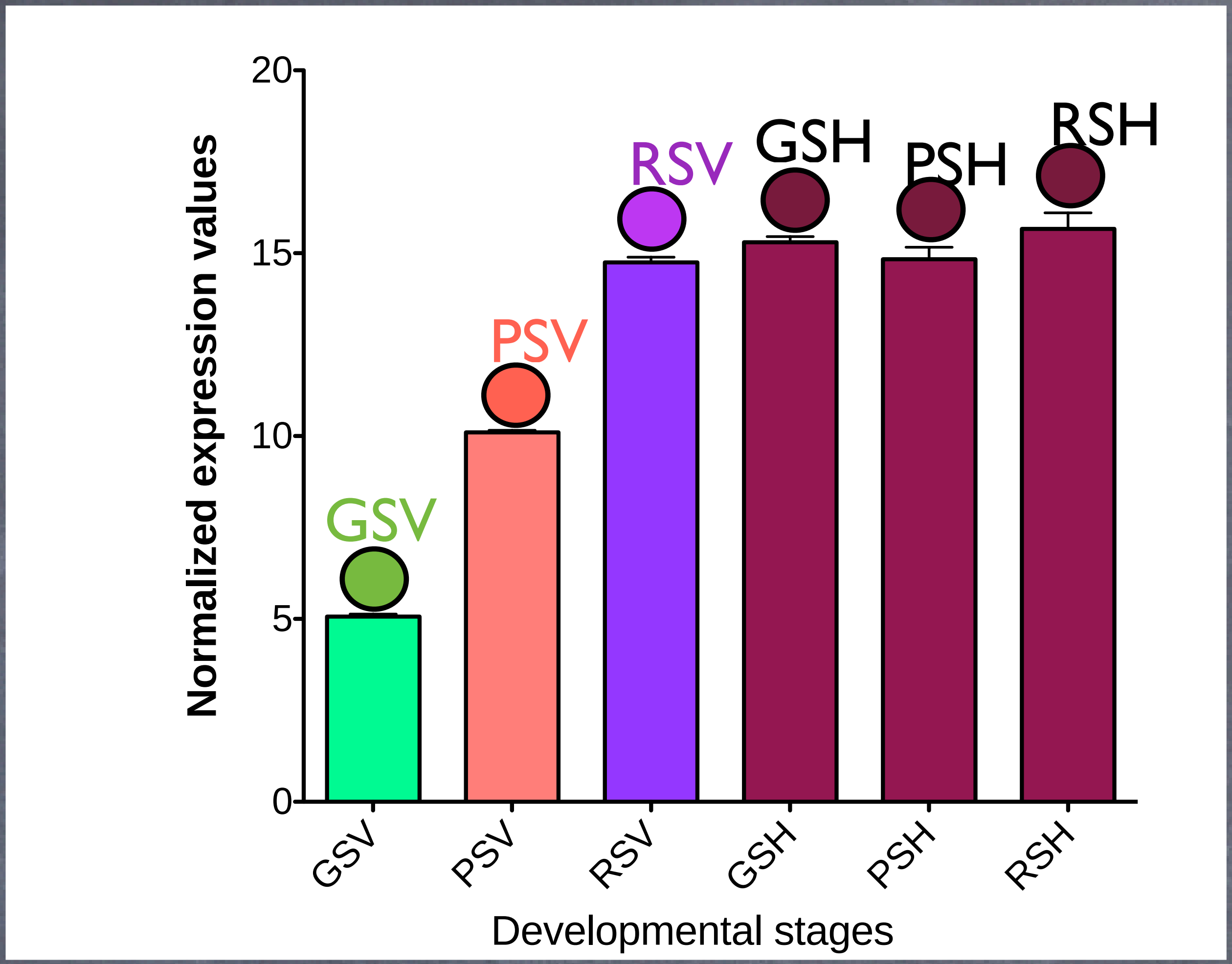

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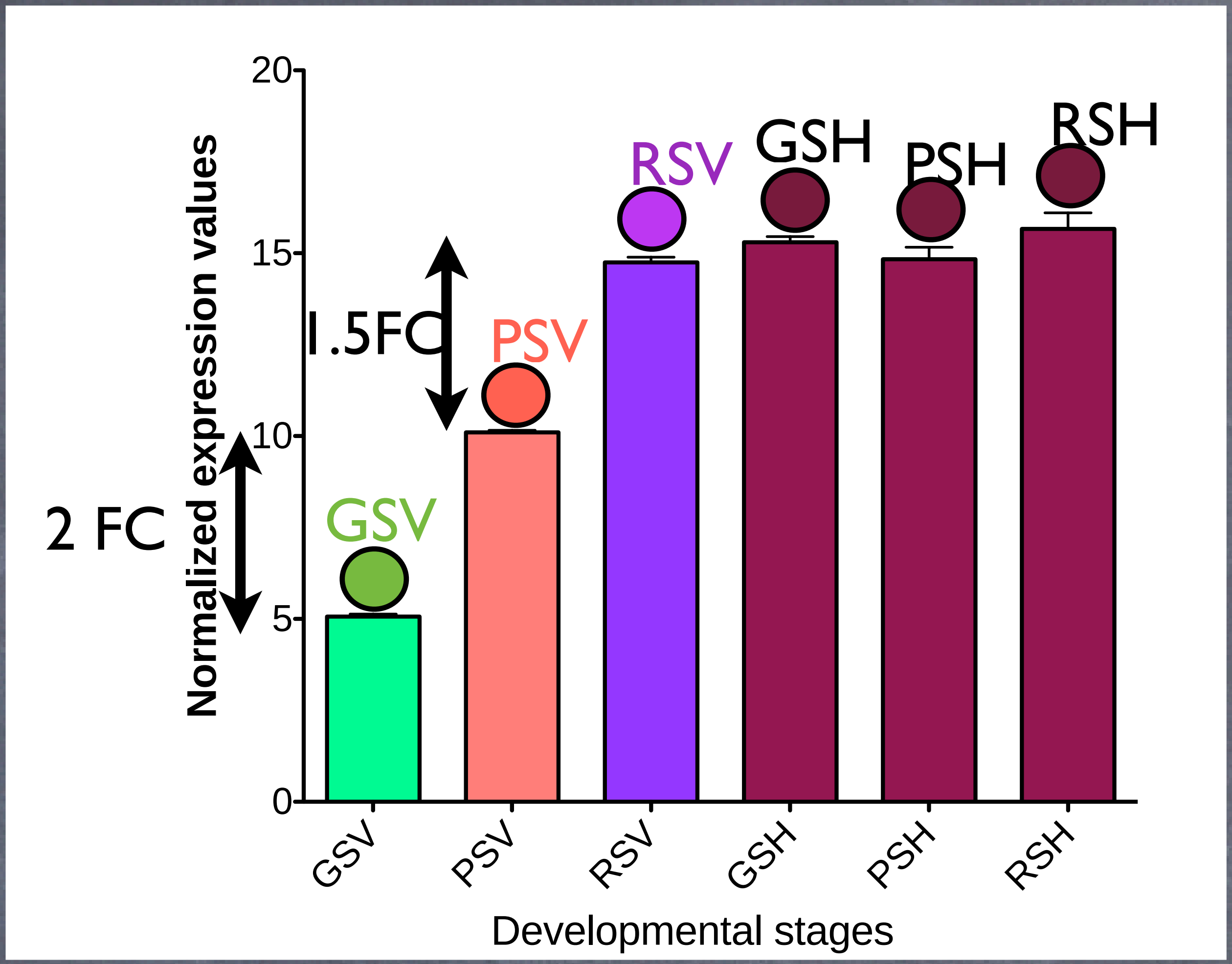

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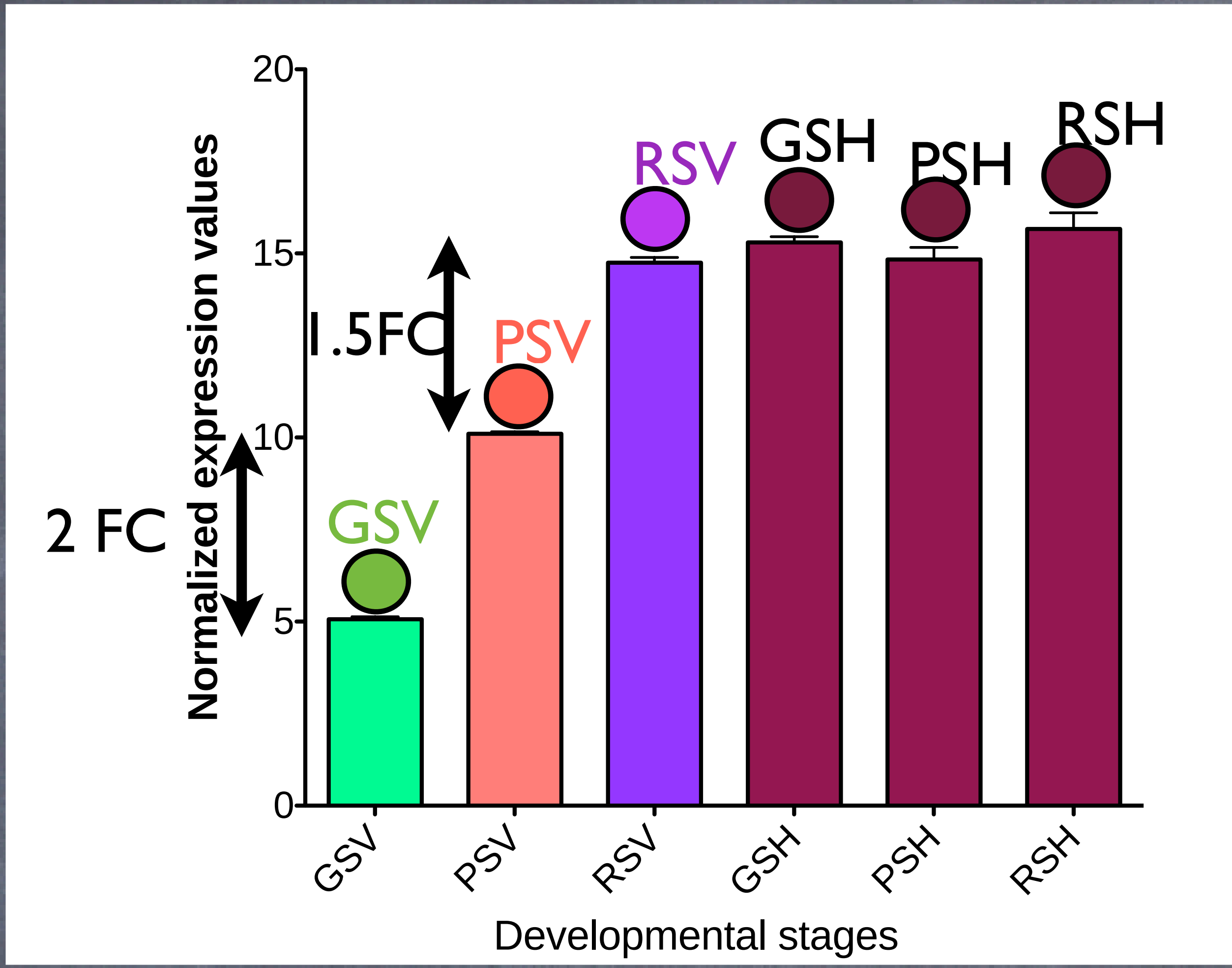

For 29,550 genes

Tissue	Mid-véraison (GSV, PSV, RSV)						Near Harvest (GSH, PSH, RSH)					
Biological significance	>1.5 Fold Change		>1.75 Fold Change		>2 Fold Change		>1.5 Fold Change		>1.75 Fold Change		>2 Fold Change	
Genes	#	%	#	%	#	%	#	%	#	%	#	%
Pulp	4,393	15	2,180	7	1,266	4	1,060	4	200	0.6	60	0.2
Seed	3,102	10	1,416	5	781	3	1,004	4	327	1.1	116	0.4
Skin	3,986	13	1,797	6	998	3	1,748	6	535	1.8	201	0.7

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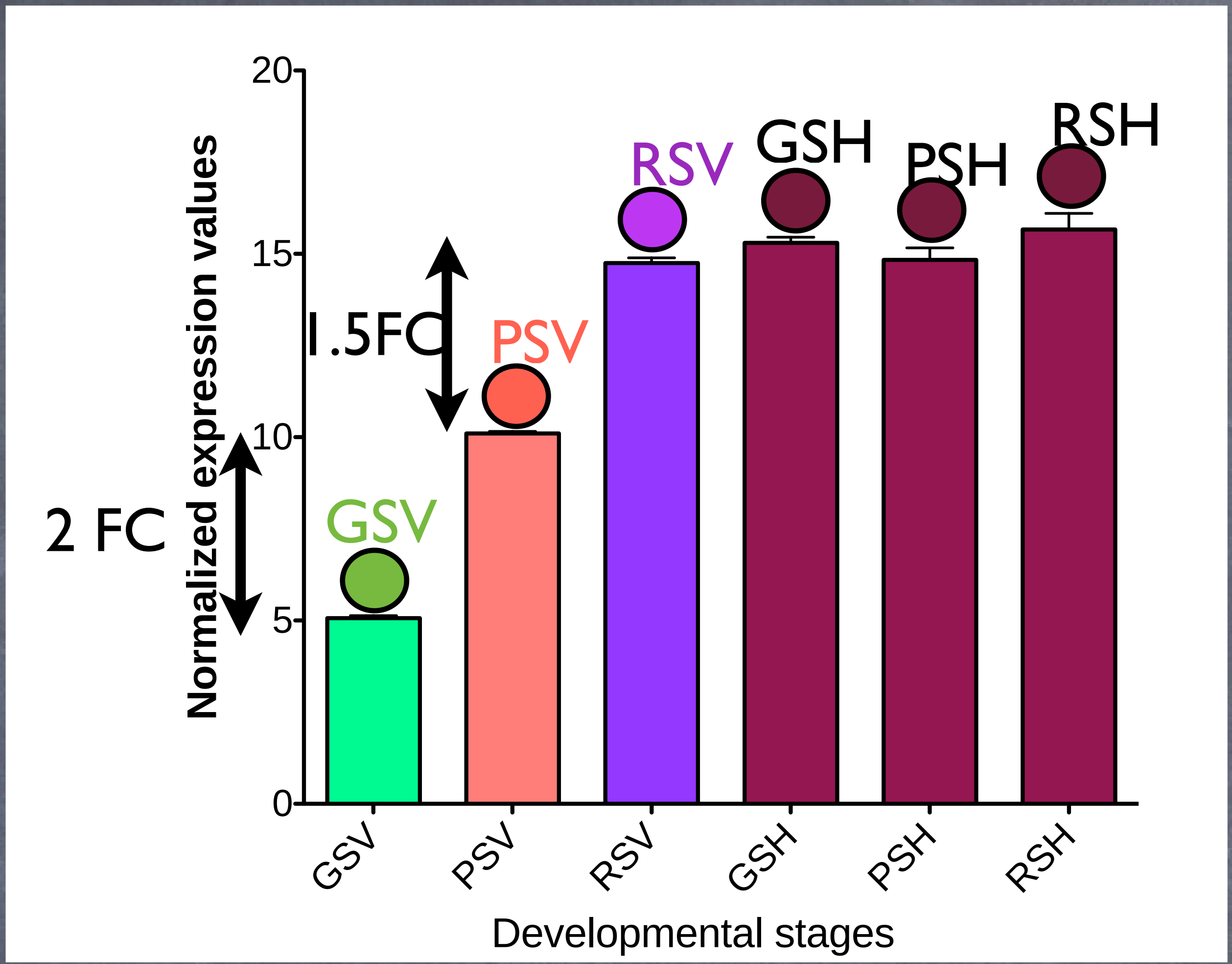

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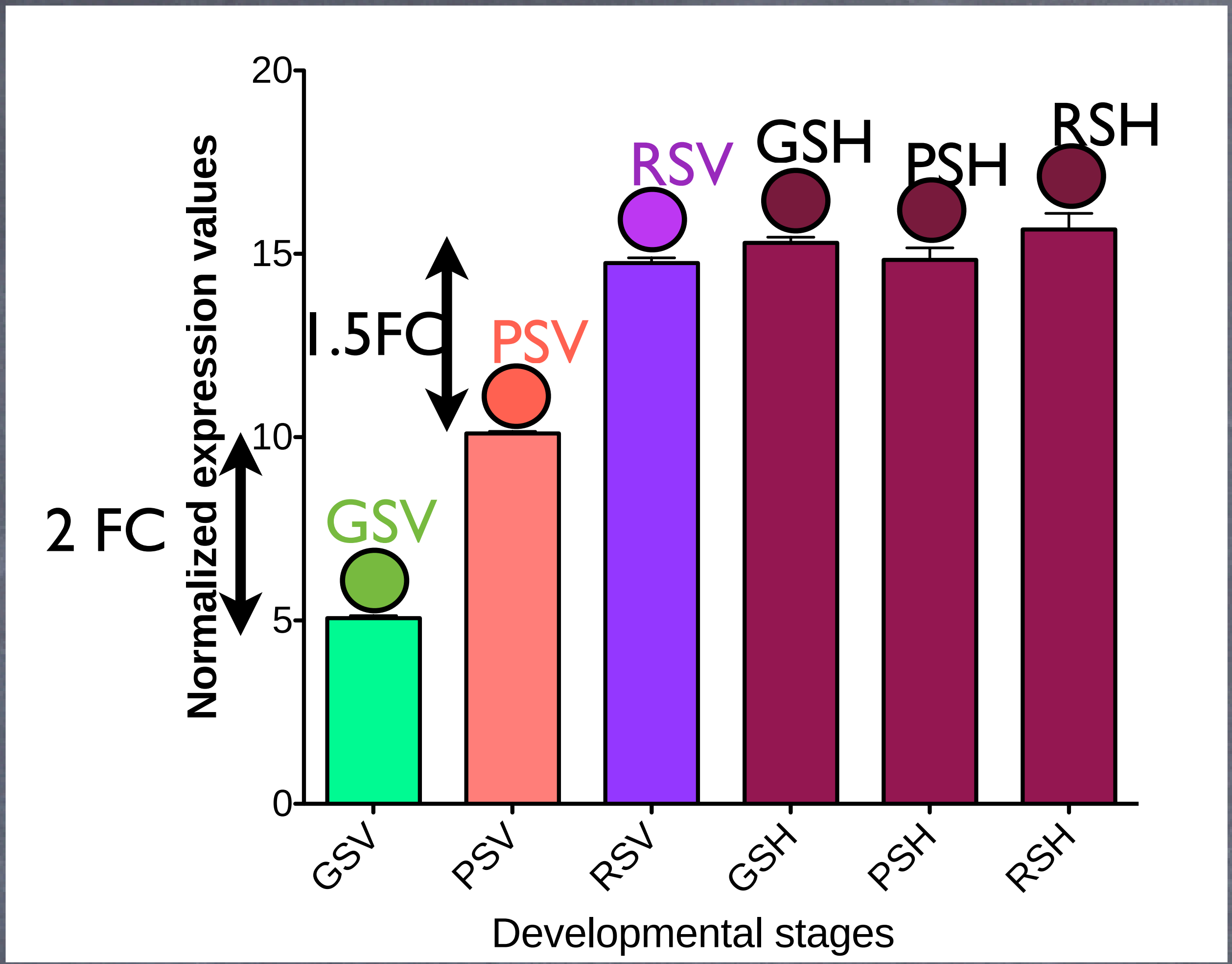

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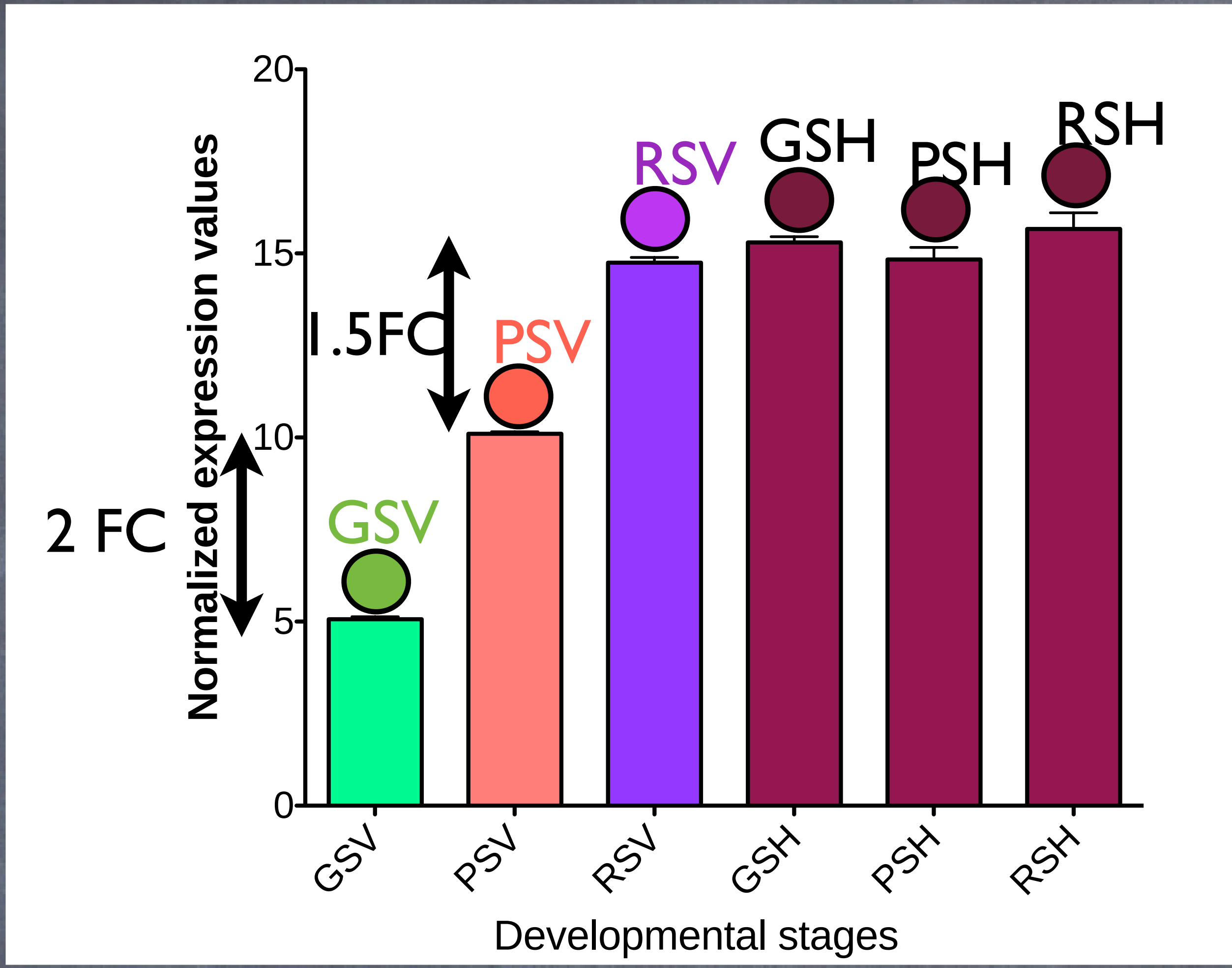

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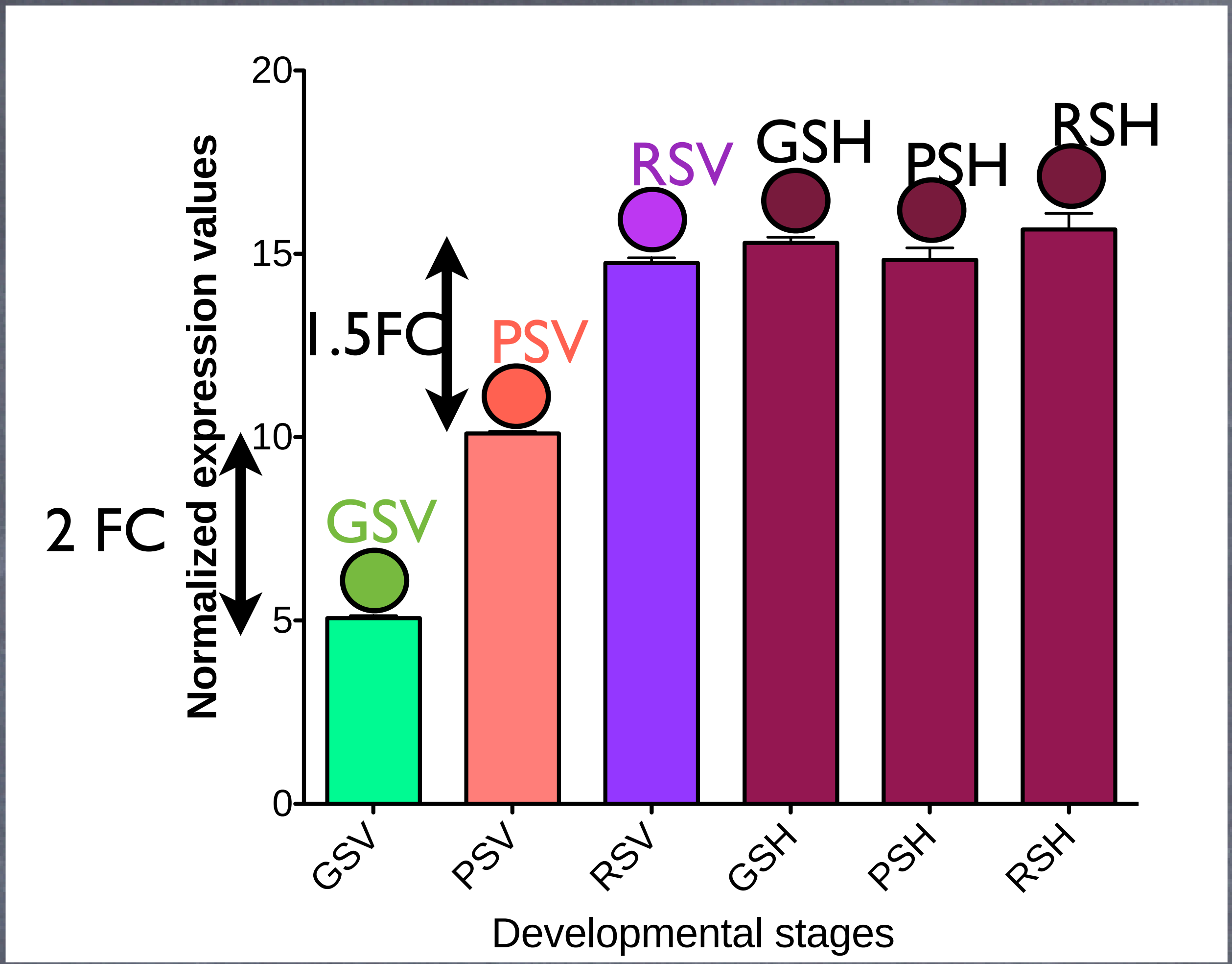

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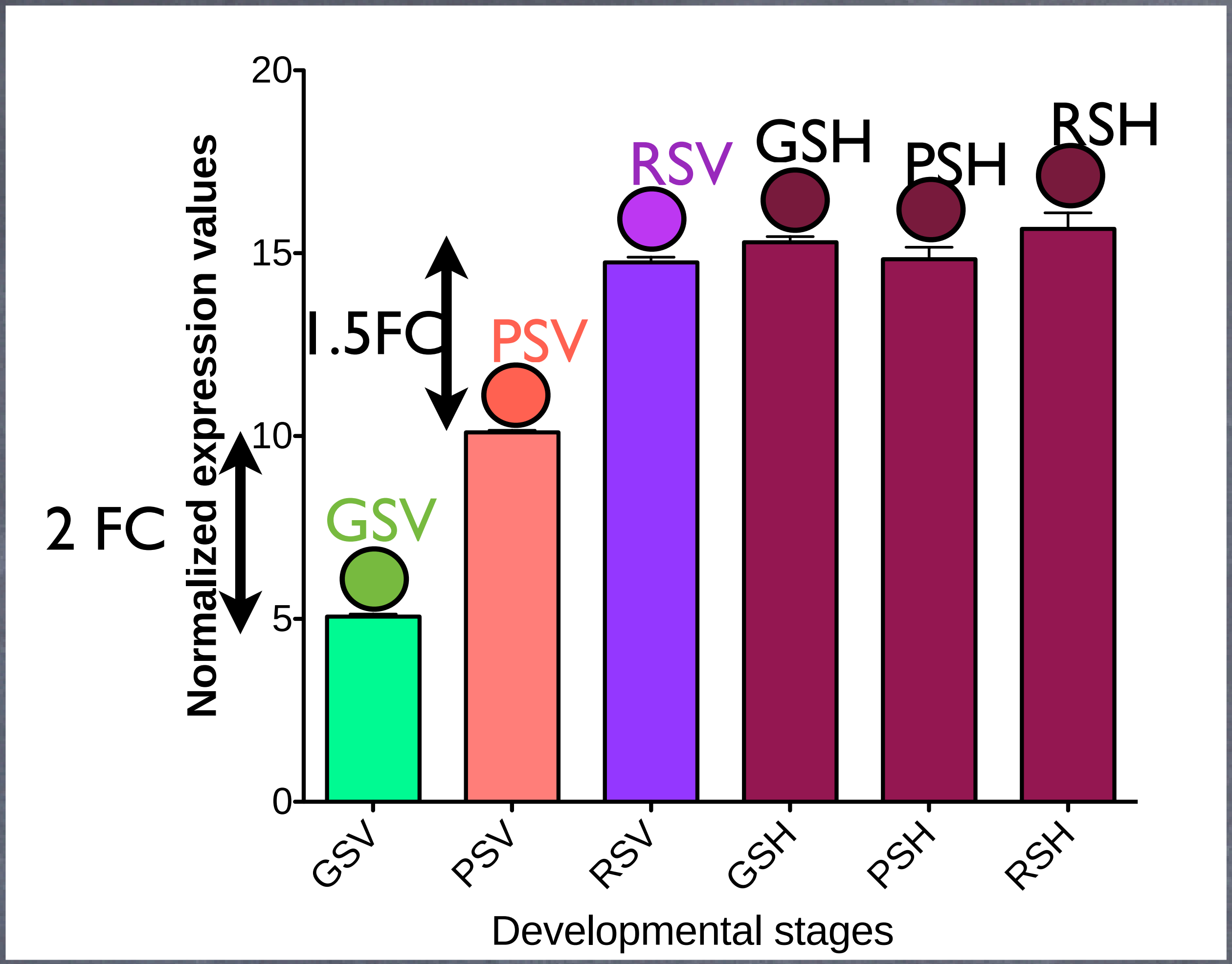

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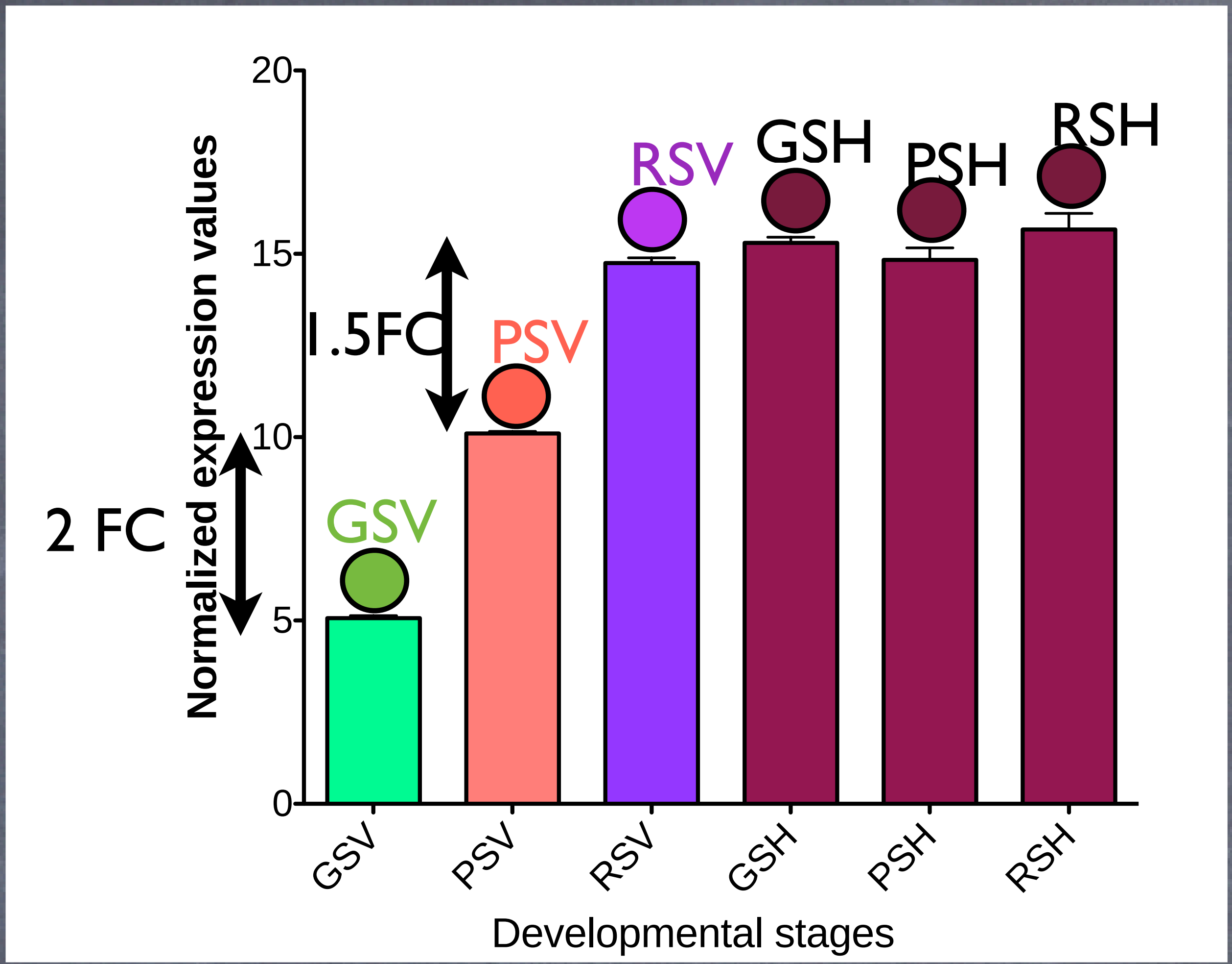

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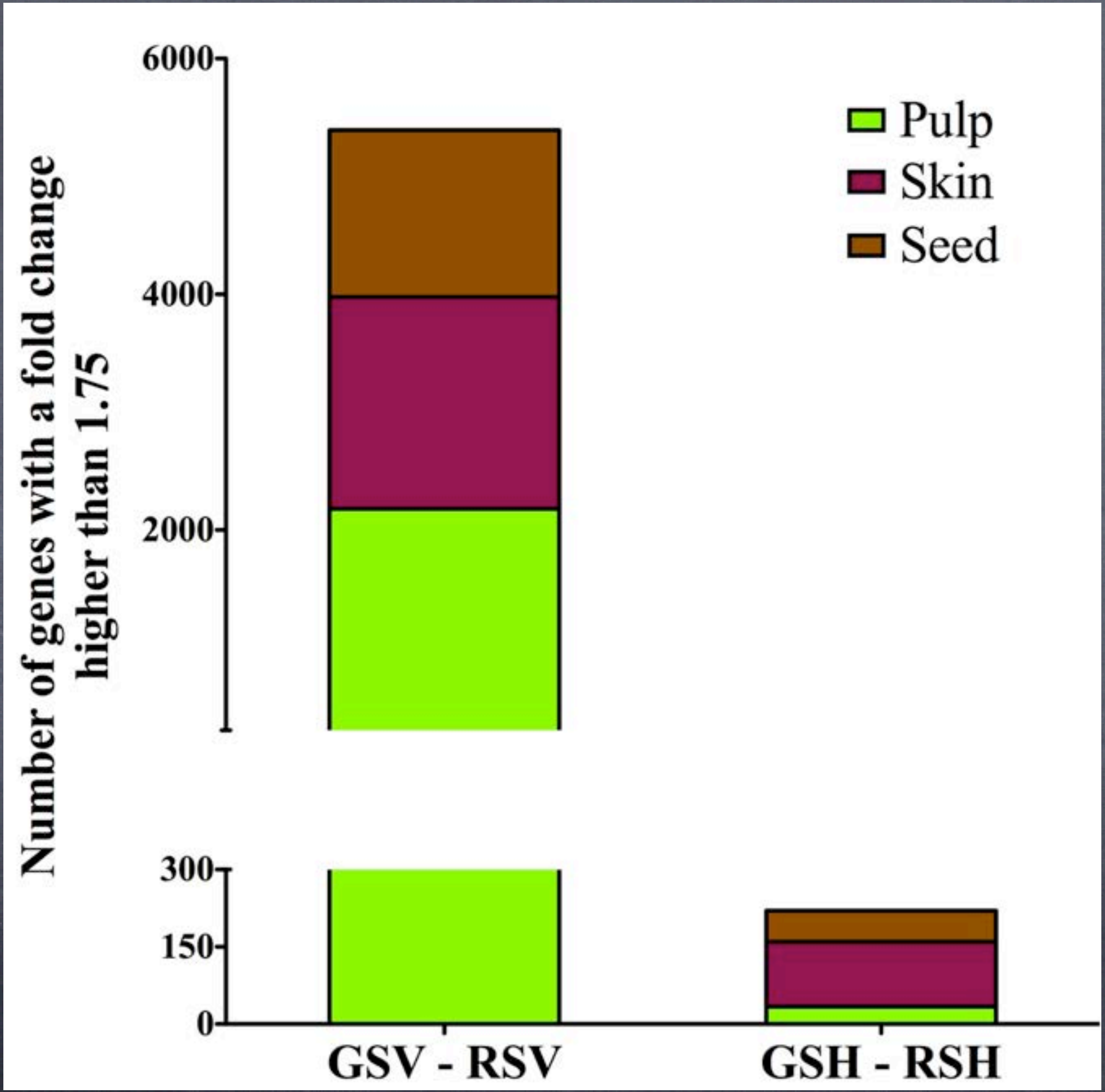


Mid-véraison Harvest

Conclusion:

Sugar and pigmentation data indicate uniform berry populations within the cluster at harvest

No perfect berry classes but more «balanced» berry classes

Reduction of berry variability at the gene expression level at harvest is effective, few genes with high variability between not fully ripe and more advanced berries

This reduction affects the three tissues

Pulp appears to be the most synchronized tissue

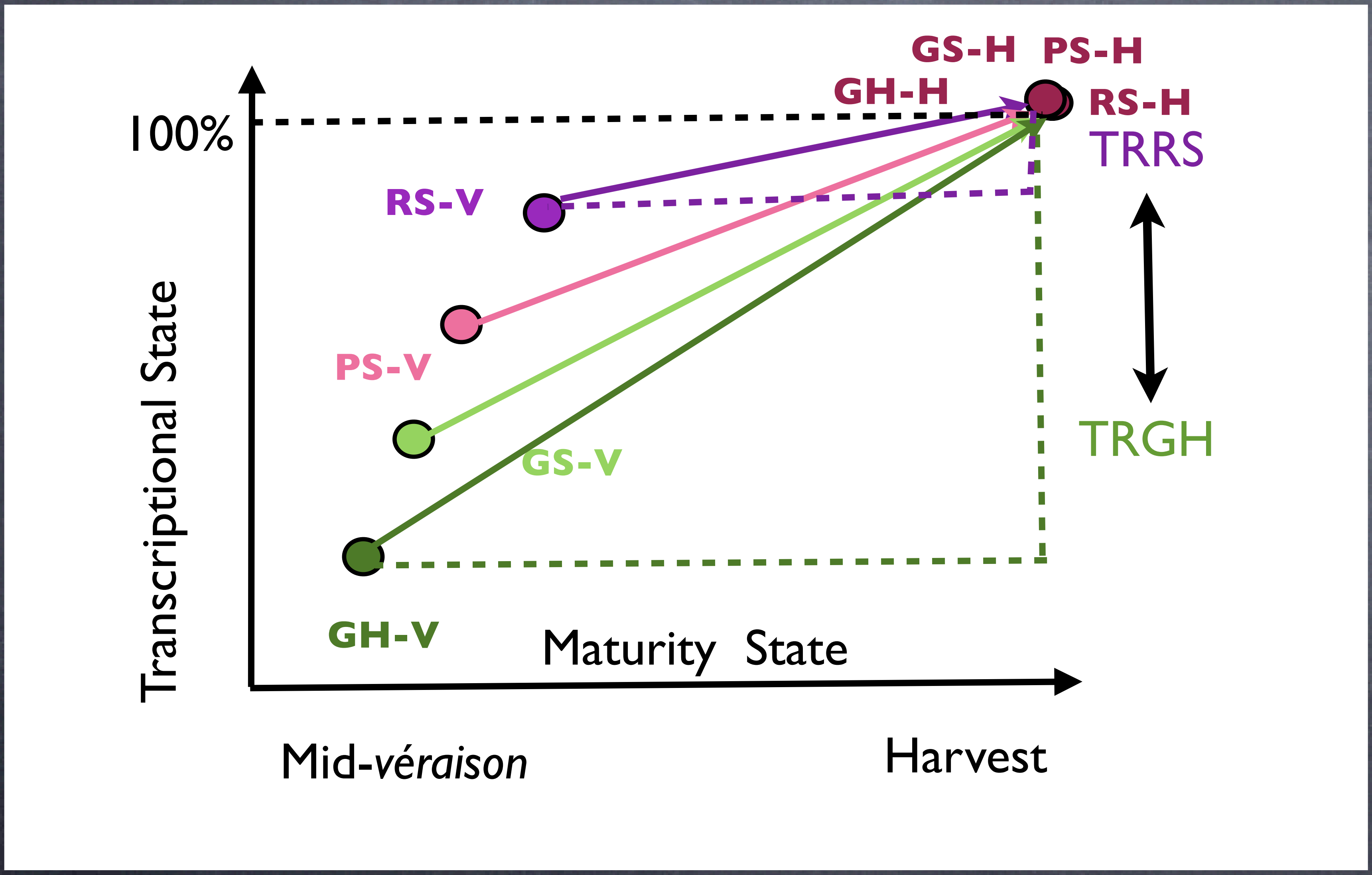
Laurent Deluc

Oregon Wine Research Institute

Oregon State University

Difference in transcriptional rates between GH and RS berry classes

Transcriptional Rate (TR): Transcriptional state at harvest - Transcriptional State at mid-véraison



TRGH: tsGHH-tsGHV

TRRS: tsRSH-tsRSV

Differential of TR:
TRGH-TRRS

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Genes in the pulp showing an enhanced transcriptional rate in GS versus RS from Mid-V to Harvest

370 genes found with an enhanced transcriptional rate superior to 2

Gene	Functional Category	Transcriptional rate (GS/RS) from V to H
Xyloglucan:xyloglucosyl transferase	Cell wall modification	3.41
Extensin	Cell wall structural protein	6.36
CYP79A2	Glucosinolate metabolism	11.96
chalcone synthase	Flavonoid biosynthesis	6.19
Flavonoid 3',5'-hydroxylase (8)	Flavonoid biosynthesis	4.14
Flavonone- 3-hydroxylase	Flavonoid biosynthesis	3.29
Chalcone isomerase	Flavonoid biosynthesis	2.49
UDP-glucose:flavonoid 3-O-glucosyltransferase	Flavonoid-glucoside biosynthesis	7.01
Caffeoyl-CoA O-methyltransferase	Phenylpropanoid biosynthesis	7.11
Limonoid UDP-glucosyltransferase	Flavor	2.46
Sugar transporter ERD6-like 16	Sugar Transporter	5.69
MATE efflux family protein	Flavonoid Transport	4.99
Anthocyanin permease AM3	Flavonoid Transport	2.25

Laurent Deluc

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Genes in the pulp showing an decreased transcriptional rate

280 genes found with a repressed transcriptional rate inferior to 0.5

Gene	Functional Category	Transcriptional rate (GS/RS) from V to H
flavonoid 3-monooxygenase	Flavonoid biosynthesis	0.4
UDP-glucosyl transferase	Flavonoid biosynthesis	0.31
flavonoid 3-monooxygenase	Flavonoid biosynthesis	0.18
UDP-rhamnose:rhamnosyltransferase	Anthocyanin-glucoside biosynthesis	0.46
Orcinol O-methyltransferase 2	Isoflavonoid biosynthesis	0.38
(+)-neomenthol dehydrogenase	Monoterpenoid biosynthesis	0.43
CYP72A1	Monoterpenoid biosynthesis	0.33

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What do we do next?

What is the extent of this reduction in berry variability at the metabolic level?

If there is a catch up, in which direction ? when the synchronization is initiated ?

Study the post-harvest stage to estimate whether the optimum of maturity is subject to variability

Characterization of some genes involved in the control of ripening

Extending this knowledge to other cultivars to understand the genetic control of ripening and the effects of environmental factors

How could these data be useful for the Industry

Validate the usefulness of these markers:

1) as descriptors and predictors of the ripening,
2) to validate the effects or the timing of viticulture practice
3) to estimate the variability not at the cluster level but at the vineyard level

Characterize the impact of berry metabolic variability in the origin of an unbalanced wine:

Development of a breeding program:

- to screen best clones that will propose uniform clusters

Laurent Deluc

Acknowledgments:

Deluc Lab:

Satyanarayana Gouthu: (Research Assistant)

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Caprice Rosato

Woodhall Station:

Alex Moeller

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