Isoprenoids

What are isoprenoids? (also called terpenoids)

Definition: ***hydrocarbons*** *structurally based on multiple isoprene units*

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Introduction:

- names: 5C isoprene derivation = terpenoids (like "turpentine")
- huge structural diversity (20,000 structures), largest group of secondary plant metabolites
- exist mostly as multiples of **5 carbons units** (the Lego principle)
- types: **hemi**, **mono**-, **sesqui**-, **di**-, **tri**-, **tetra-terpenes** (5, 10, 15, 20, 30, 40 carbons)

  also polyterpenes (very large)
- two complementary biosynthetic routes are known, for different groups of isoprenoids

  i) **mevalonate** (MVA) pathway

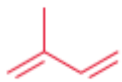
  ii) **deoxyxylulose phosphate** pathway

- impressive **functional diversity**: many **volatile** compounds, signals, toxins, hormones and more.
- some compounds form this family function as primary metabolites, but most fall into the secondary metabolite category
- many human uses of isoprenoids from or in plants (fragrances, flavors, pharmaceuticals, rubber)

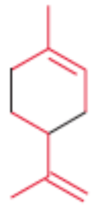
Strategy and Outline

- learn basic biosynthesis pathways of isoprenoid building blocks.
- examples with specific functions of each class of isoprenoids

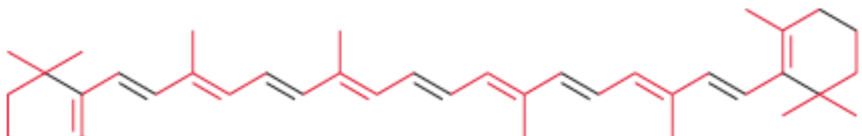
Introduction: Representative isoprenoid structures



Isoprene



Limonene,
a monoterpene



β-Carotene,
a tetraterpene

Rubber,
a polyterpene

Introduction: Functional diversity of isoprenoids

Table 17.1: Isoprenoids of higher plants

Precursor	Class	Example	Function
C_5: Dimethylallyl-PP	Hemiterpene	Isoprene	Protection of the photosynthetic apparatus against heat
Isopentenyl-PP		Side chain of cytokinin	Growth regulator
C_{10}: Geranyl-PP	Monoterpene	Pinene	Defense substance attractant
		Linalool	
C_{15}: Farnesyl-PP	Sesquiterpene	Capsidiol	Phytoalexin
C_{20}: Geranylgeranyl-PP	Diterpene	Gibberellin	Plant hormone
		Phorbol	Defense substance
		Casbene	Phytoalexin
C_{30}: 2 Farnesyl-PP	Triterpene	Cholesterol	Membrane constituents
		Sitosterol	
C_{40}: 2 Geranylgeranyl-PP	Tetraterpene	Carotenoids	Photosynthesis pigments
n Geranylgeranyl-PP or ***n*** Farnesyl-PP	Polyprenols	Prenylated proteins	Regulation of cell growth
		Prenylation of plastoquinone, ubiquinone, chlorophyll, cyt ***a***	Membrane solubility of photosynthesis pigments and electron transport carriers
		Dolichols	Glucosyl carrier
		Rubber	

Overview of Isoprenoid Biosynthesis

- two complementary biosynthetic routes are known, for different groups of isoprenoids

i) **mevalonate** (MVA) pathway

ii) **deoxyxylulose phosphate** pathway

i. Mevalonate pathway

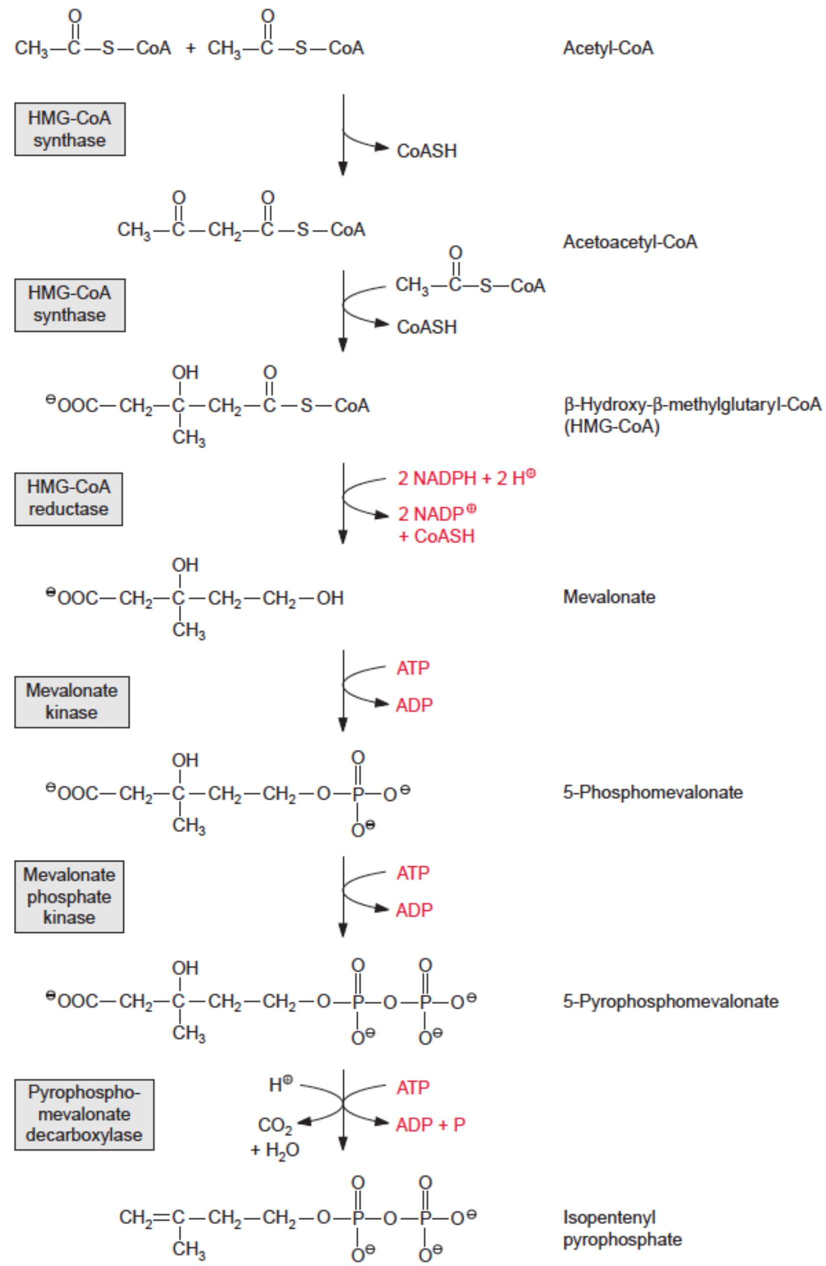
→ produces **isopentenyl pyrophosphate** (IPP) for sesqui- and tri-terpenes

Hydroxymethylglutaryl CoA (HMGCoA) synthase (key gateway enzyme)

- hydroxymethylglutaryl CoA (HMGCoA) reductase

- kinases, dehydratases

- IPP isomerase makes methylallyl pyrophosphate (DMAPP)



Deoxyxylulose phosphate (DXP) [Methylerythriol (MEP) pathway]

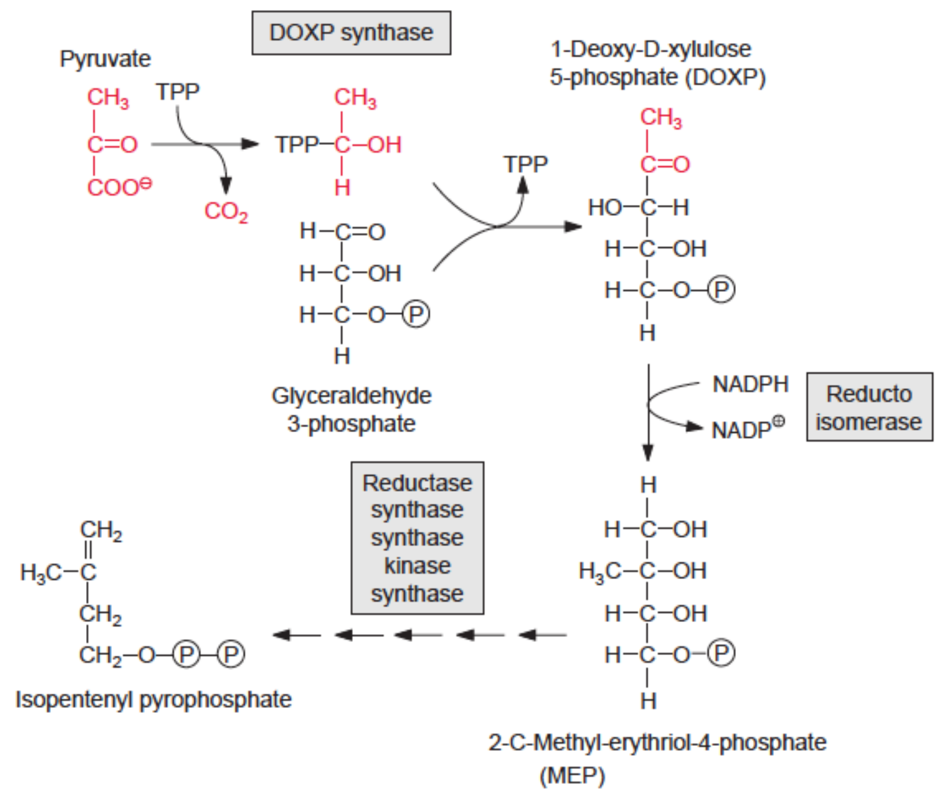
→ produces isopentenyl pyrophosphate (IPP)

ii). Deoxyxylulose phosphate (**DXP**) pathway

- in **plastids**,
- leads to mono-, di-, and tetra-terpenes
- also found in bacteria, some protists, algae

Precursors: **pyruvate** & **glyceraldehyde-3-P**

- products: both IPP and DMAPP
- key enzyme: *deoxyxylulose phosphate (DXP) synthase*



Step 2: Successive condensations of **IPP** with **DMAPP** to form larger isoprenoids

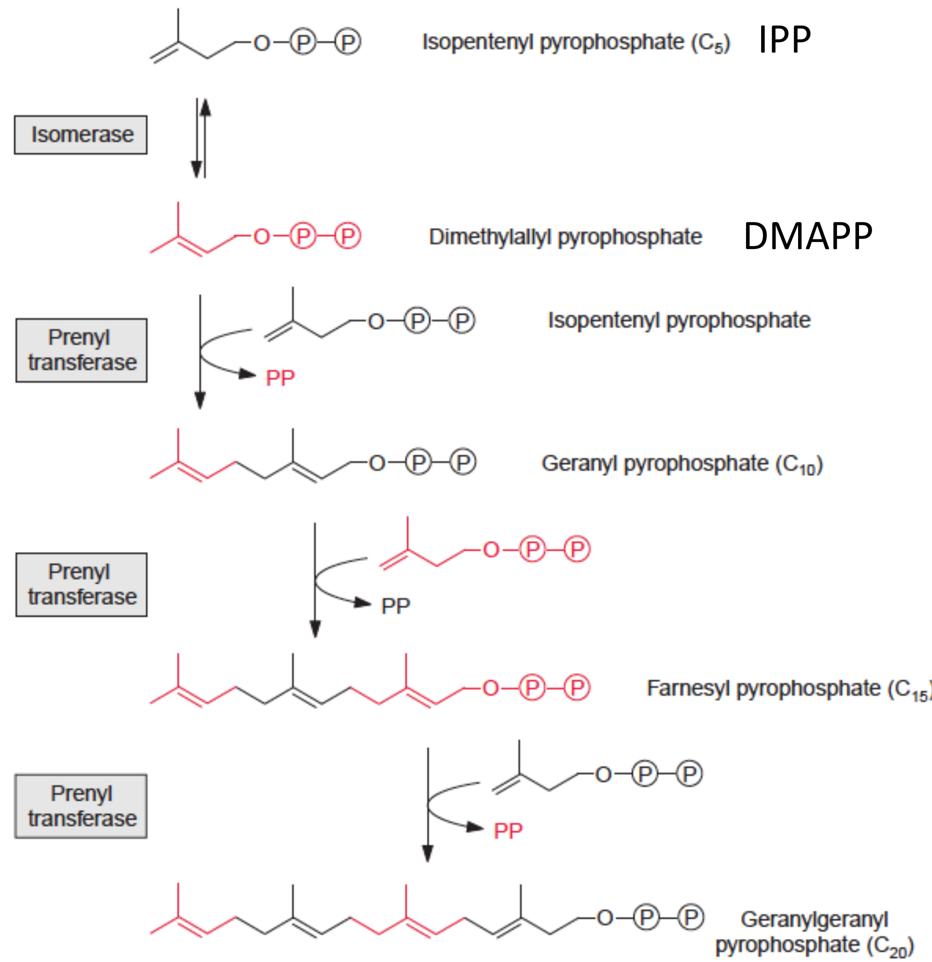
- head-to-tail ('lego')
- enzymes: *prenyl transferases*

Note: one enzyme does multiple condensations, but is specific for each end product

1 IPP + 1 DMAPP --> **geranyl-PP**
geranyl-PP synthase

2 IPP + 1 DMAPP --> **farnesyl-PP**
farnesyl-PP synthase (one enzyme)

3 IPP + 1 DMAPP --> **geranylgeranyl-PP**
geranylgeranyl-PP synthase (one enzyme)



Step 2: Successive condensations of IPP with DMAPP to form larger isoprenoids

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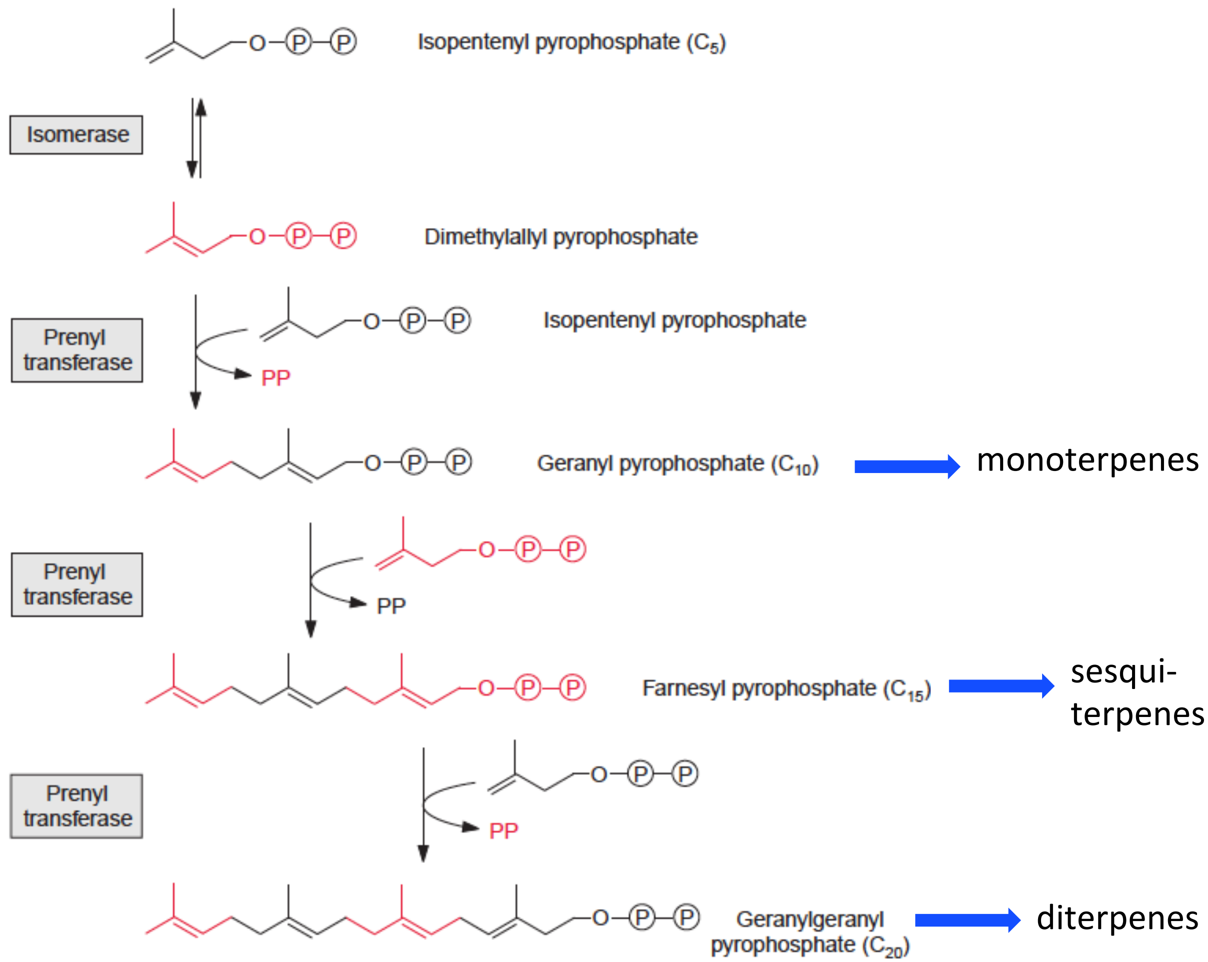


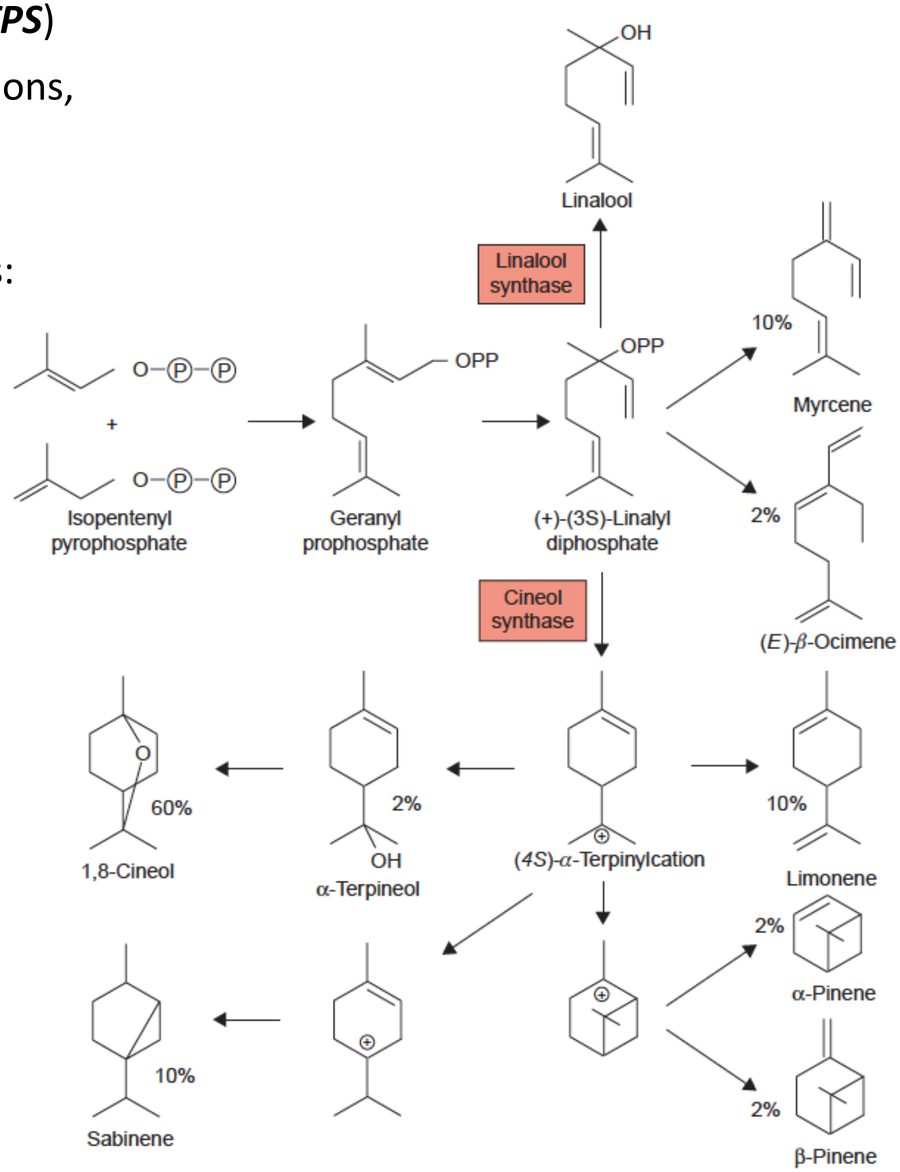
Step 3:   Further elaborations and modifications - monoterpenes

- cyclizations (***terpene synthases*** - ***TPS***)
- hydroxylations, oxidations, reductions, rearrangements

Example of monoterpene synthases:

- **linalool synthase** (specific)
- **cineol synthase** ('sloppy')

  -> multiproduct enzyme



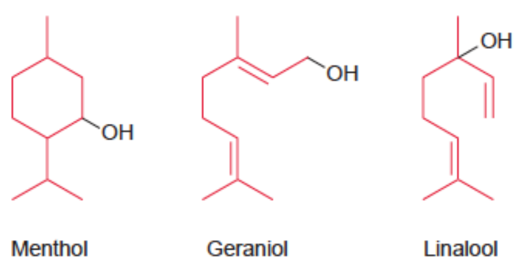


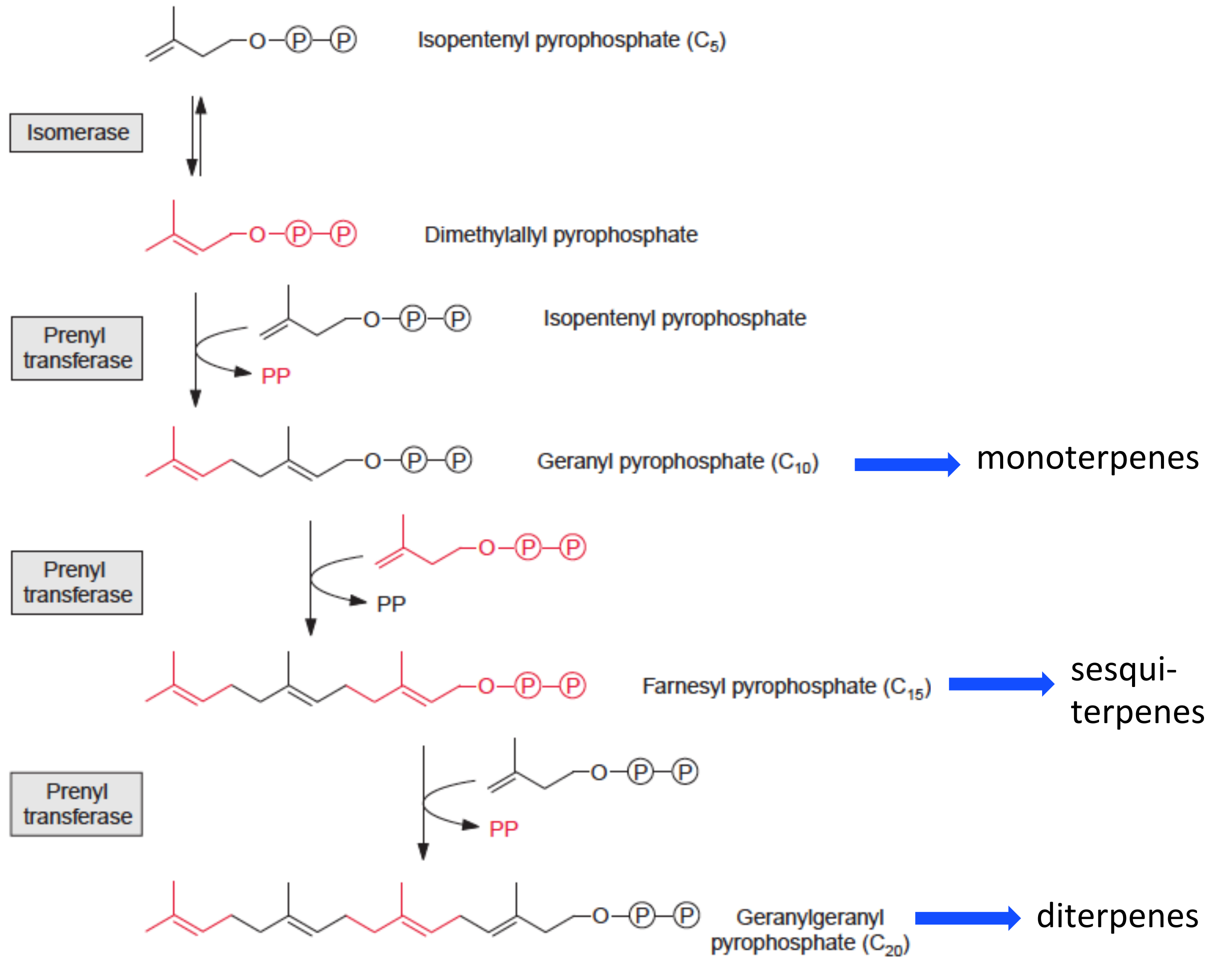
Fig 17.8, 9

Step 2: Successive condensations of IPP with DMAPP to form larger isoprenoids

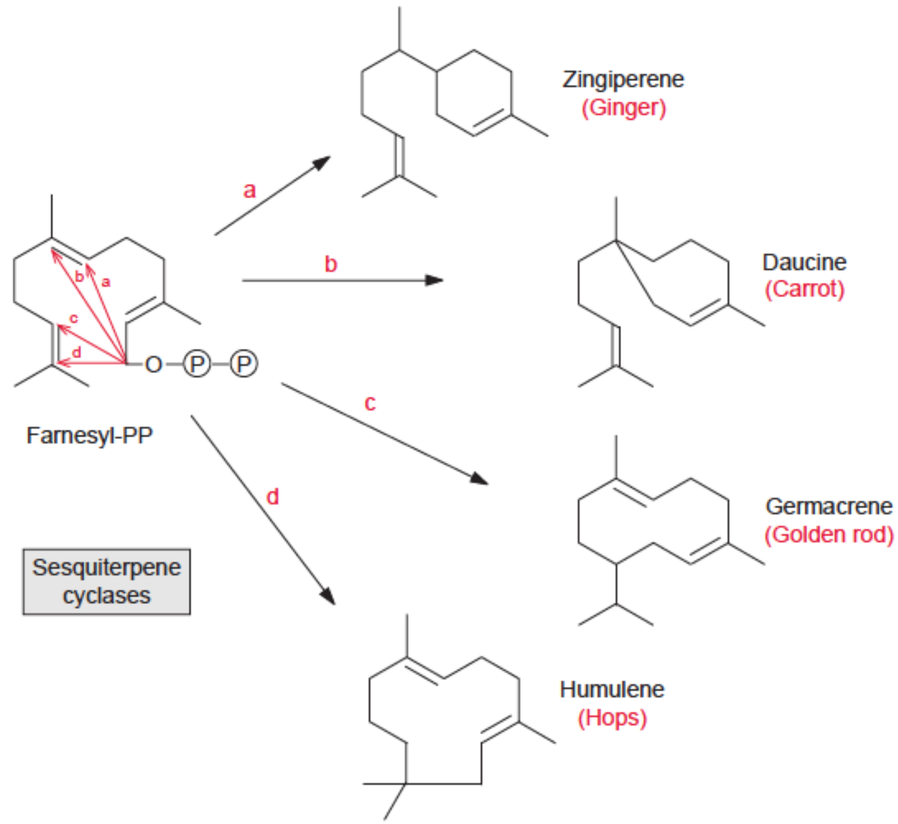
- head-to-tail ('lego')

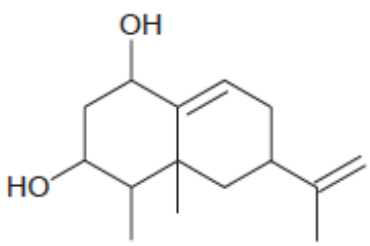
- enzymes: *prenyl transferases*

Note: one enzyme does multiple condensations, but is specific for each end product



Step 3: Further elaborations and modifications – sesquiterpene synthase (cyclase)



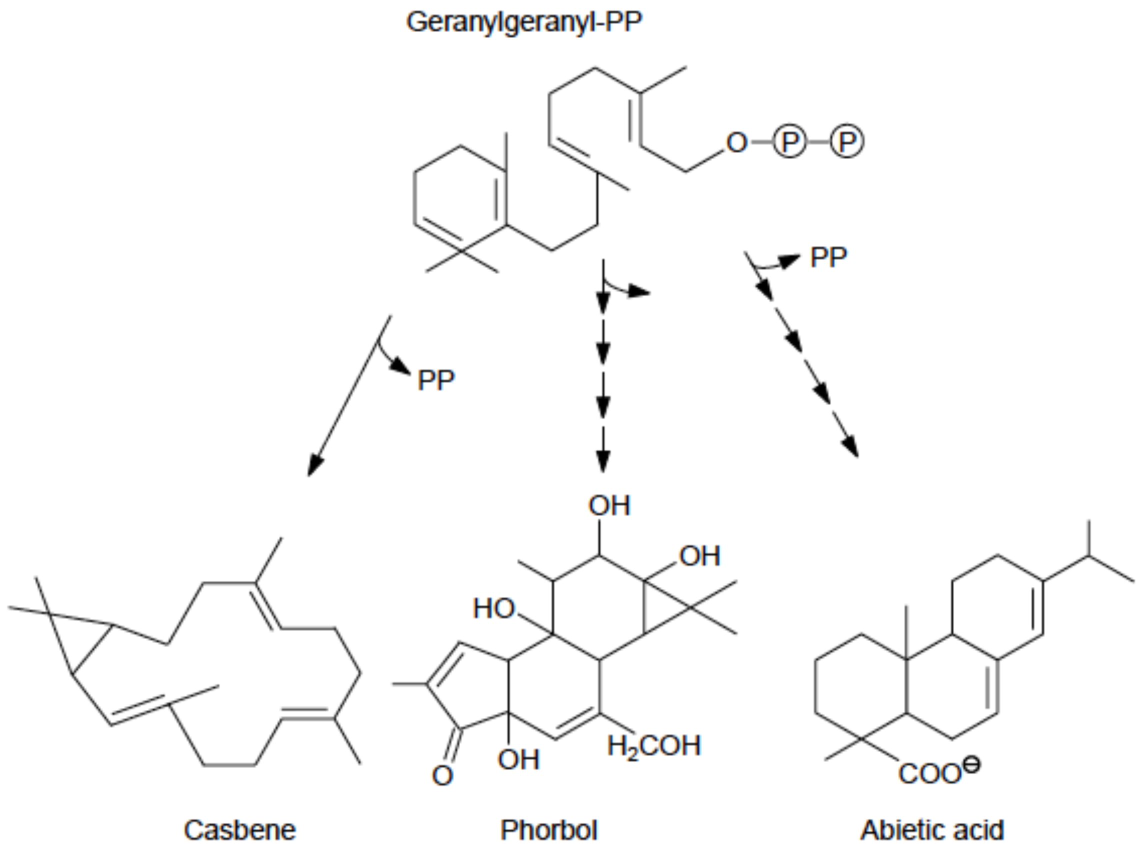


Capsidiol

Fig 17.10

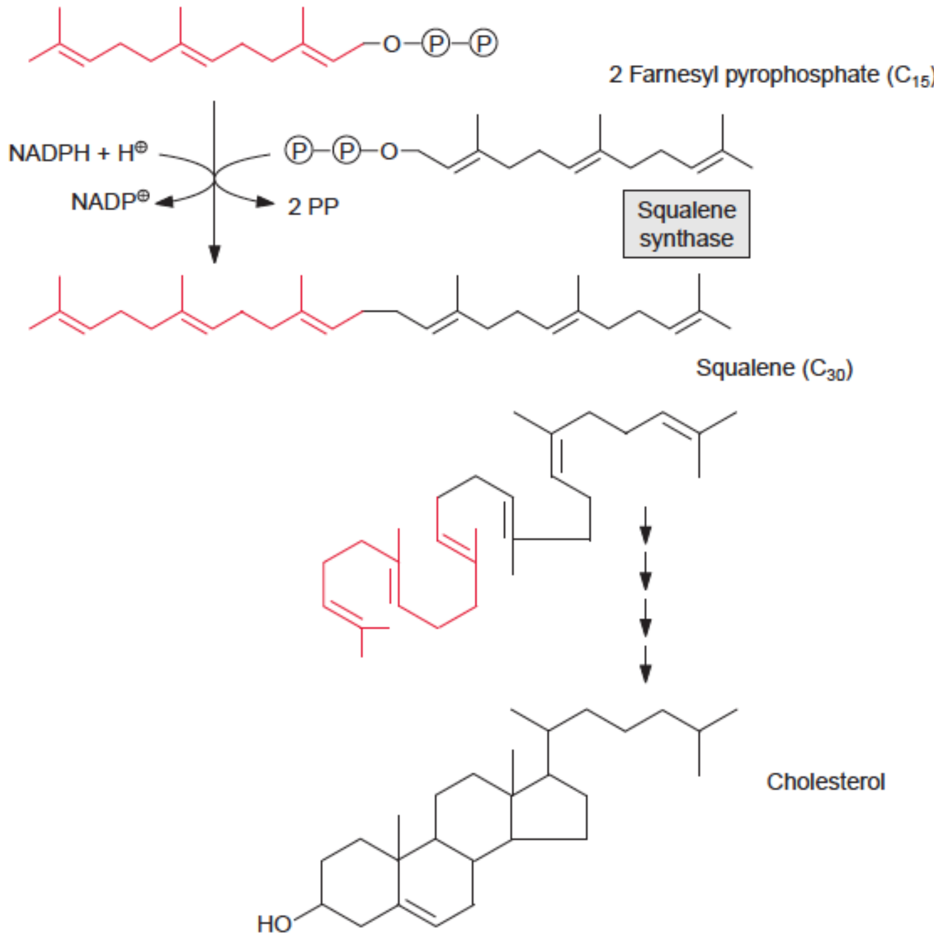
Step 3: Further elaborations and modifications - diterpenes

Fig 17.13



Step 4: Further condensations to triterpenes

Fig 17.11

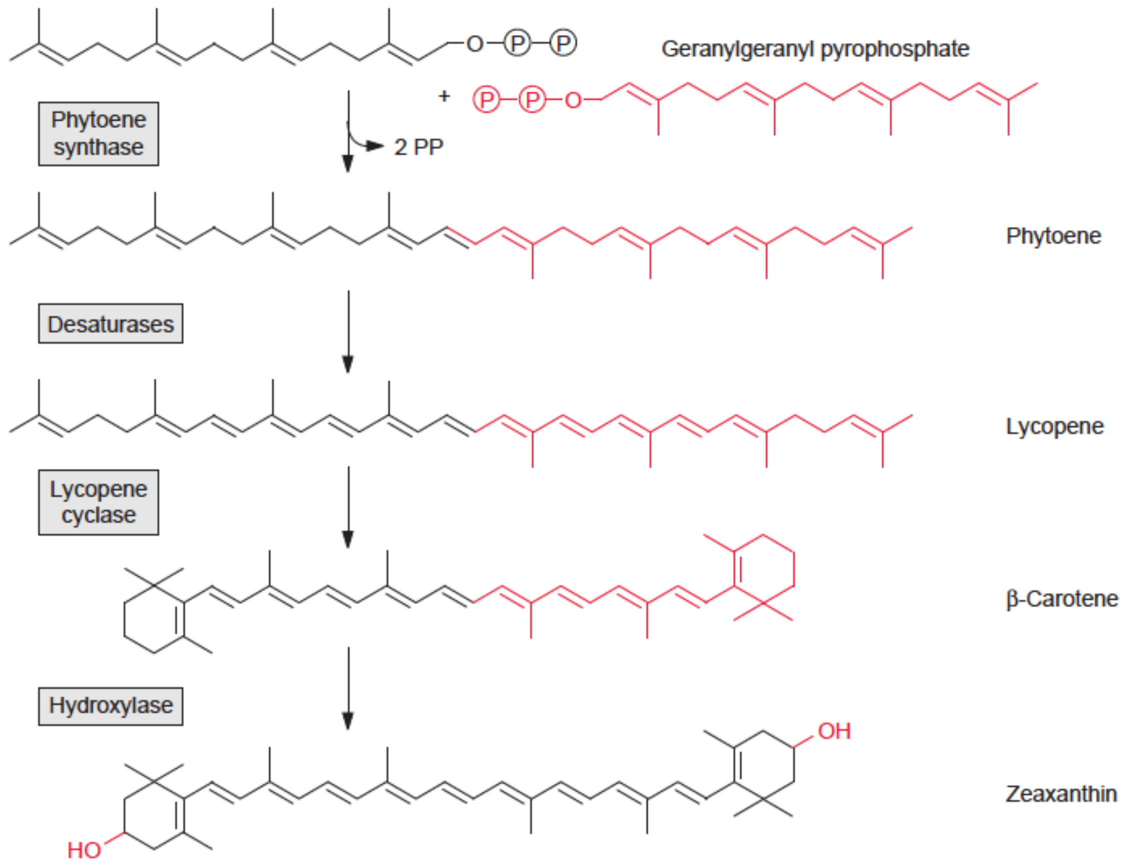


2 x farnesyl-PP --> triterpenes (sterols and steroids, cardiac glycosides, others)

key enzyme: squalene synthase

Step 3: Further condensations - tetraterpenes

Fig 17.14

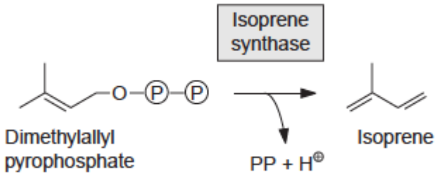


2 x geranylgeranyl-PP --> tetraterpenes (carotenoids, xanthophylls and derived products

enzyme: phytoene synthase

[redacted]

Hemiterpenes: Isoprene emission from leaves of trees (heat-stressed)



www.atmos.colostate.edu/ ~heald/research.html

Transgenic (non-isoprene emitting) poplar plants are more heat-sensitive

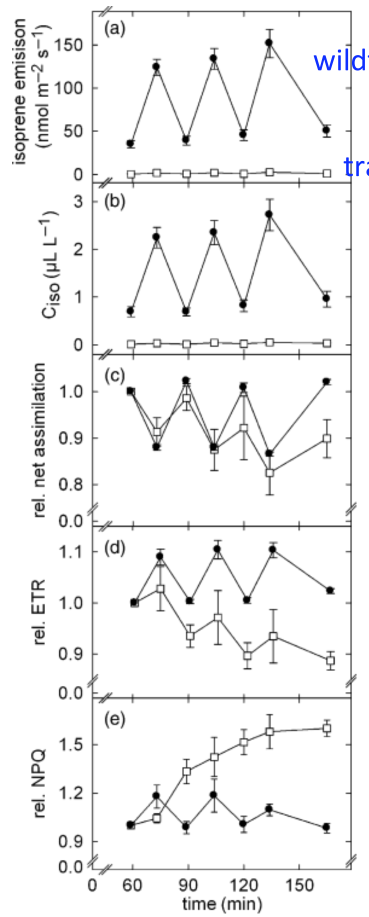
Leaf isoprene emission

Leaf isoprene concentration

net assimilation

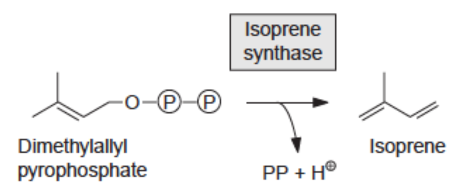
photosynthetic electron transport

non-photochemical quenching



wildtype plant

transgenic (isoprene-nonemitters)



The Plant Journal
Volume 51, Issue 3, pages 485-499, 21 JUN 2007 DOI: 10.1111/j.1365-313X.2007.03157.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1365-313X.2007.03157.x/full#f5

2. Functions for monoterpenes and sesquiterpenes

i) **direct defenses** (toxic/repellent)

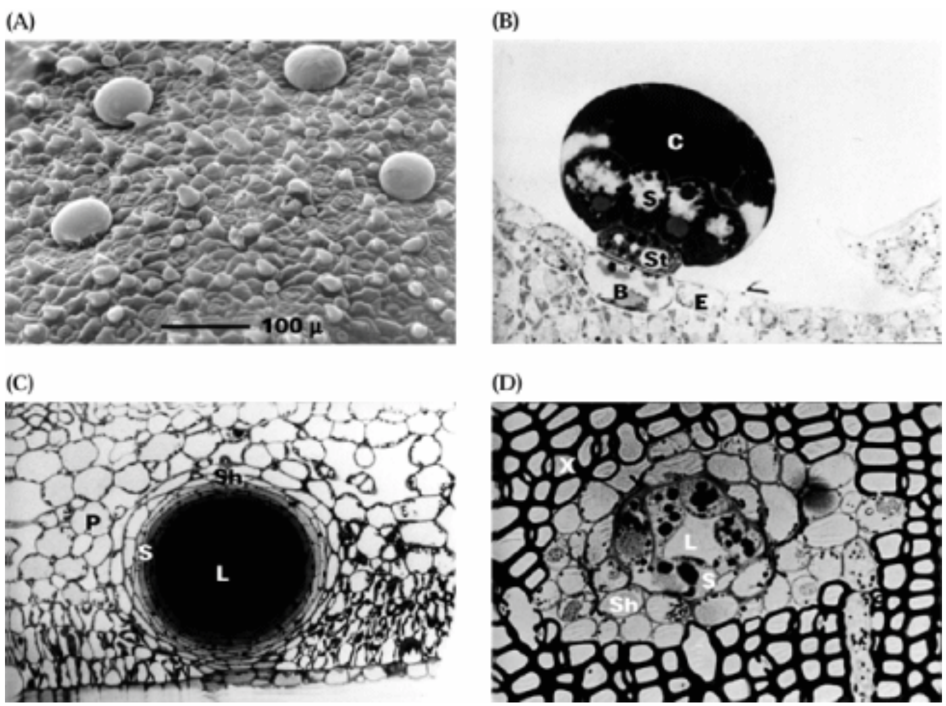
*P*eppermint trichomes (special modified hairs) contain monoterpenes and sesquiterpenes

- released by herbivores (insects) breaking trichome

Structures that accumulate (lipophilic) monoterpenes

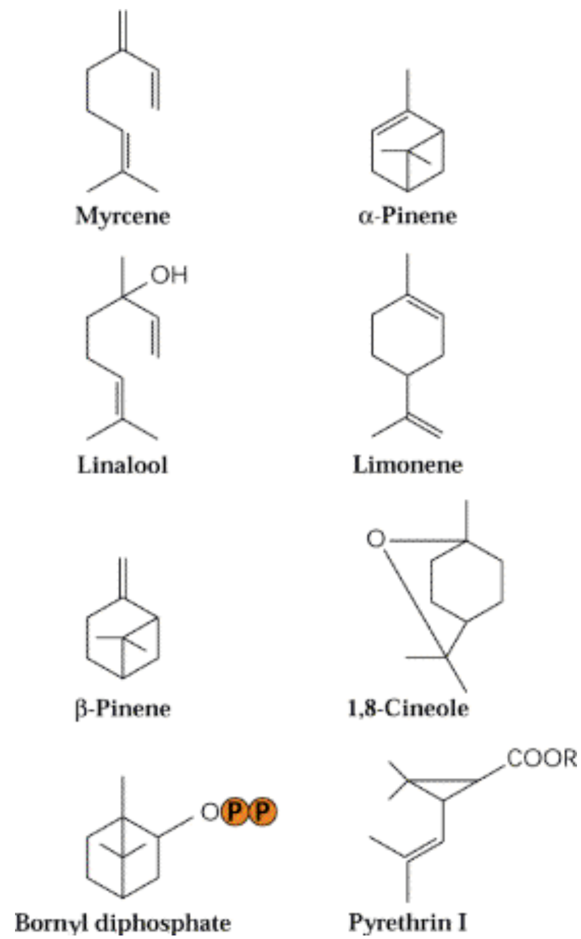
glands (thyme)

glandular trichomes (spearmint)



secretory cavity (lemon leaf)

resin duct (Jeffrey pine)



iii) Many mono- or sequiterpenes are **volatile** (= ***ecological signals***)

- attract pollinators, seed dispersors (see scents and fragrances
  (eg) linalool, limonene (recall: methyl benzoate, a phenolic volatile)
- attract **predators** (=act as **indirect defenses**)

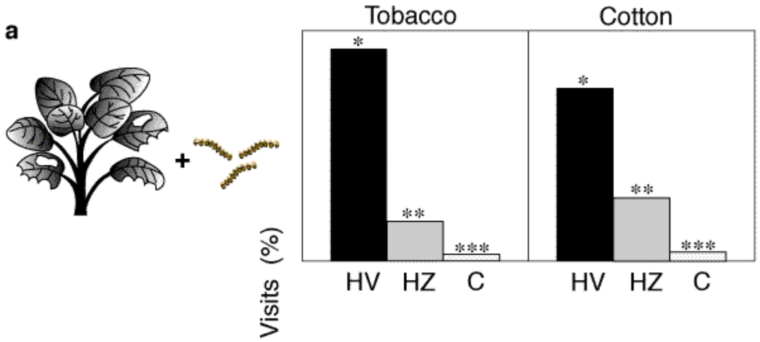
www2.unine.ch/entomo/page24332_en_US.html

Parasitic wasps attacking Spodoptera on maize leaf

Parasitoid pupae
on lepidopteran pest

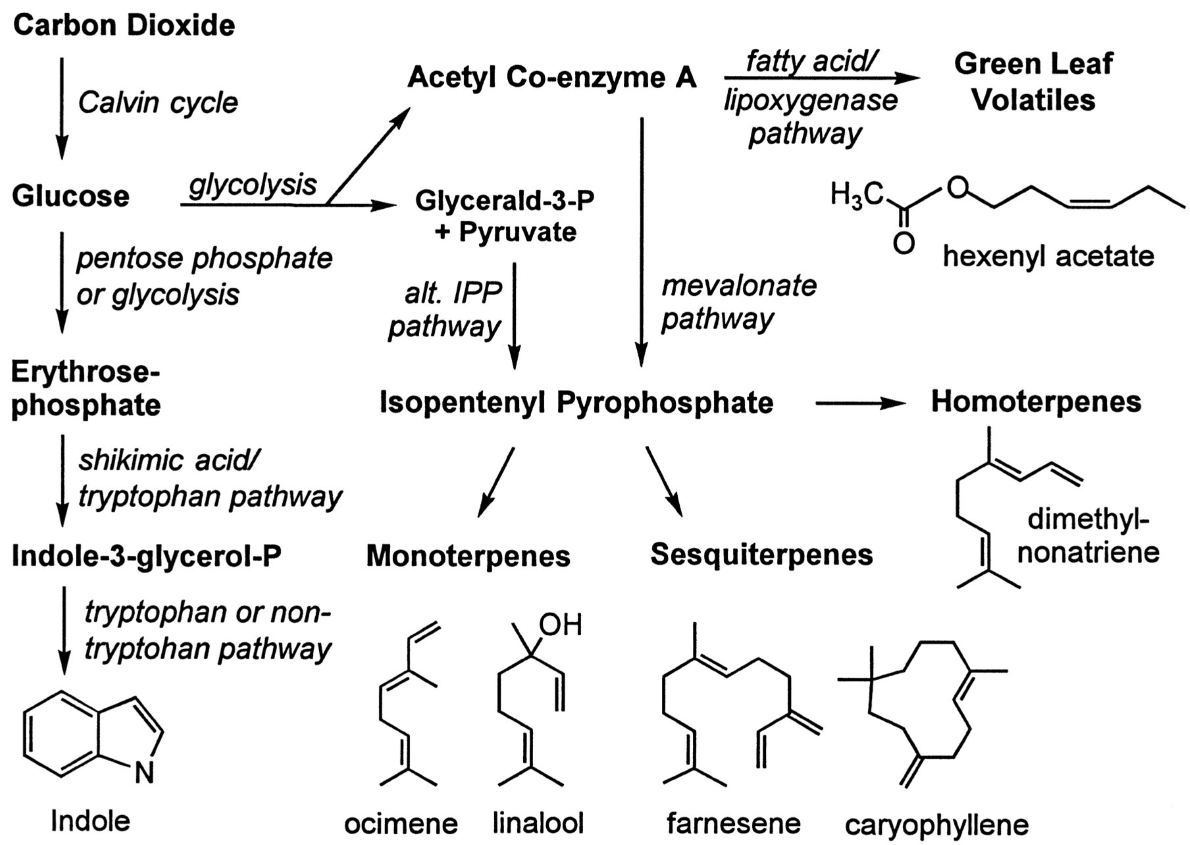
Mono- and sesquiterpenes: volatile cues for parasitic wasps and predators

Cardiochiles nigriceps (parasitic wasp) searching for *Heliothis virescens* (caterpillar)



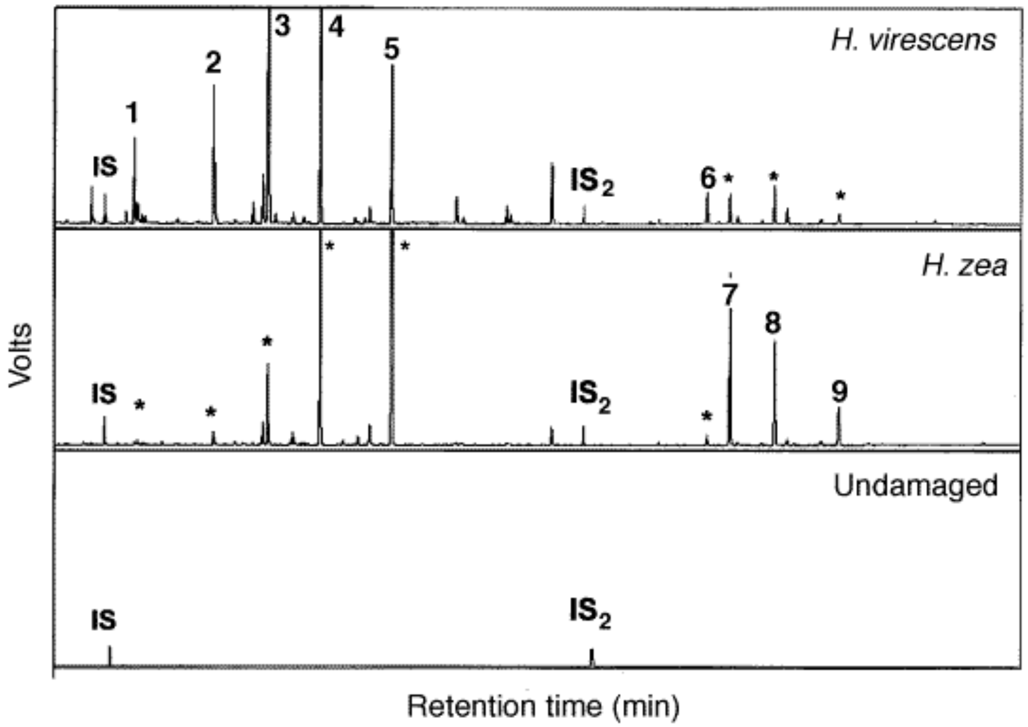
(De Moraes et al, Nature 393: 570)

Biosynthetic pathways leading to the release of plant volatiles

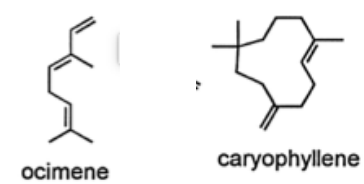


Par P. W., Tumlinson J. H. Plant Physiol. 2010:121:325-332

Gas chromatograph comparing damage by two types of caterpillars



(De Moraes et al, Nature 393: 570)

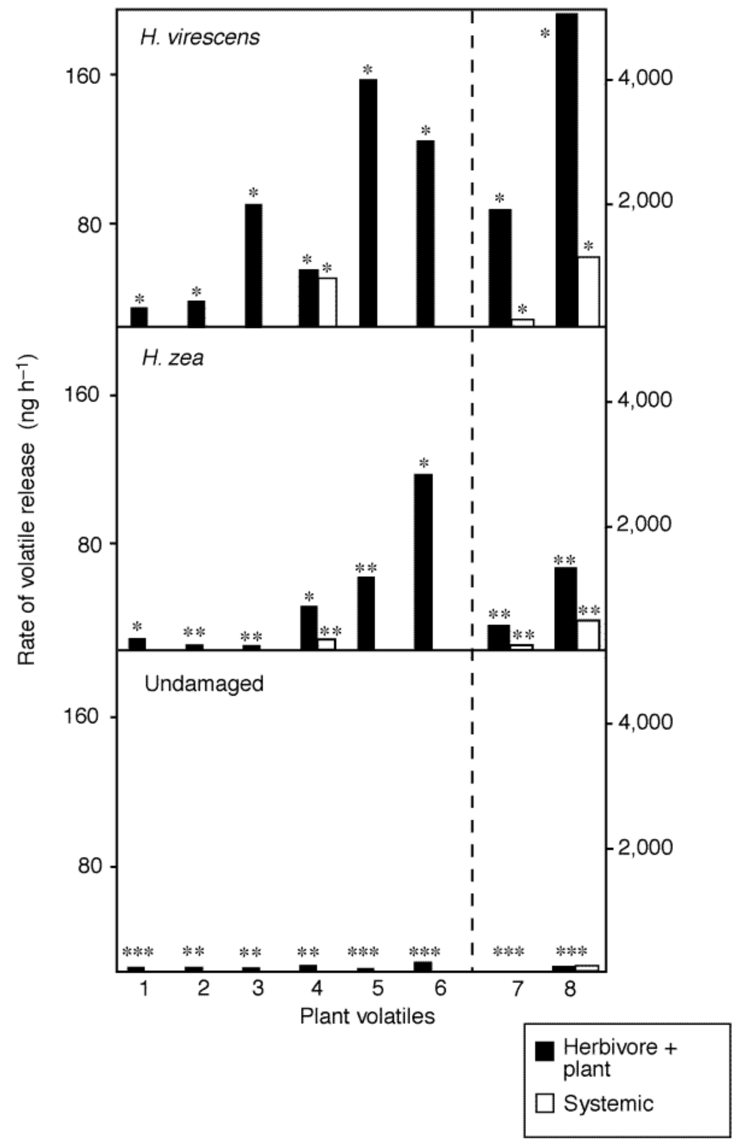


(7) ß-0cimene

(8) a-0cimene

(9) ß-caryophyllene

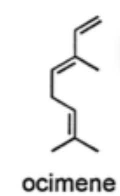
Gas chromatograph comparing damage by two types of caterpillars



(De Moraes et al,

Nature 393: 570)

(7) ß-0cimene
(8) a-0cimene



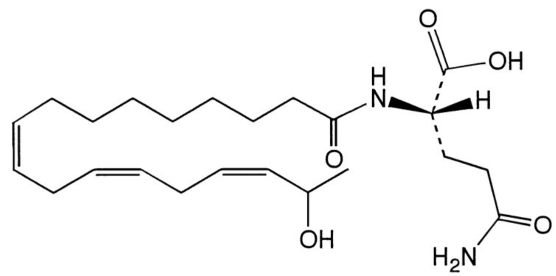
What is the basis of differences in release of volatiles from H zea and H virescens?

-> the biochemistry of caterpillar regurgitant or "spit"….

What is the basis of differences in release of volatiles from H zea and H virescens?

An Elicitor of Plant Volatiles from Beet Armyworm Oral Secretion

H. T. Alborn, T. C. J. Turlings,* T. H. Jones, G. Stenhagen
J. H. Loughrin,† J. H. Tumlinson‡



Volicitin

N-(17-hydroxylinolenoyl)-L-glutamine

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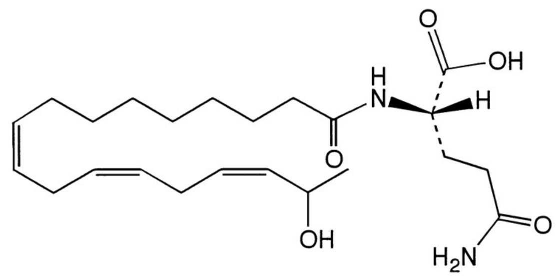
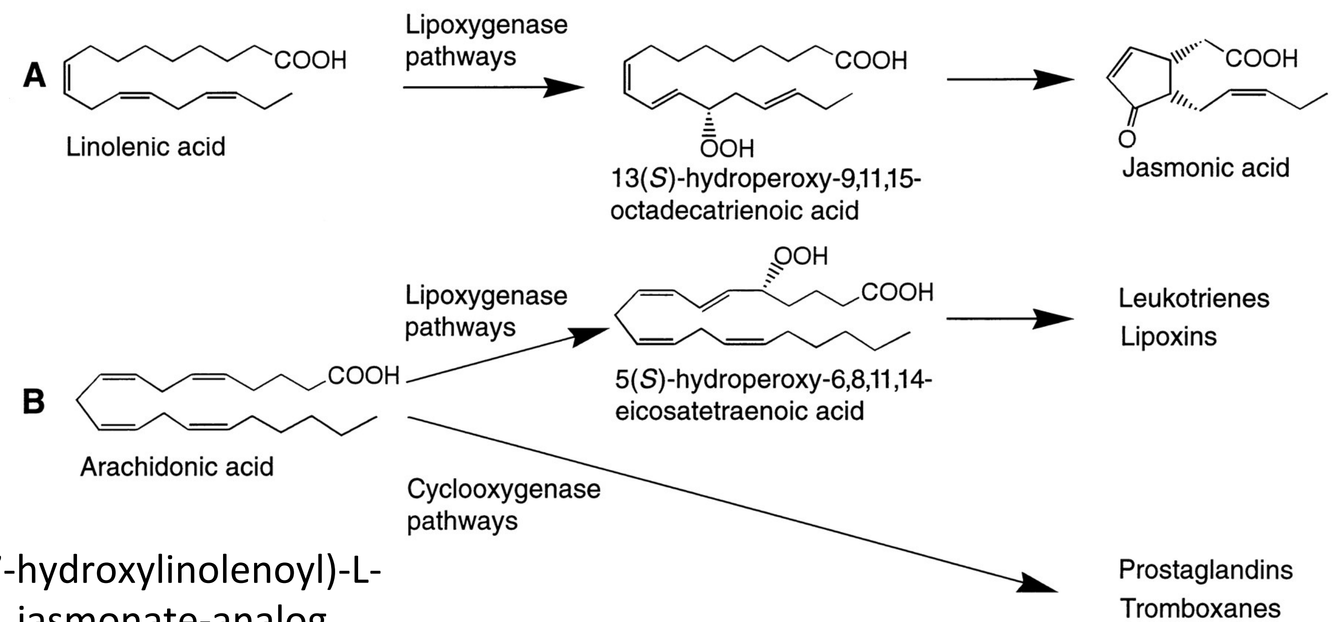


Volicitin

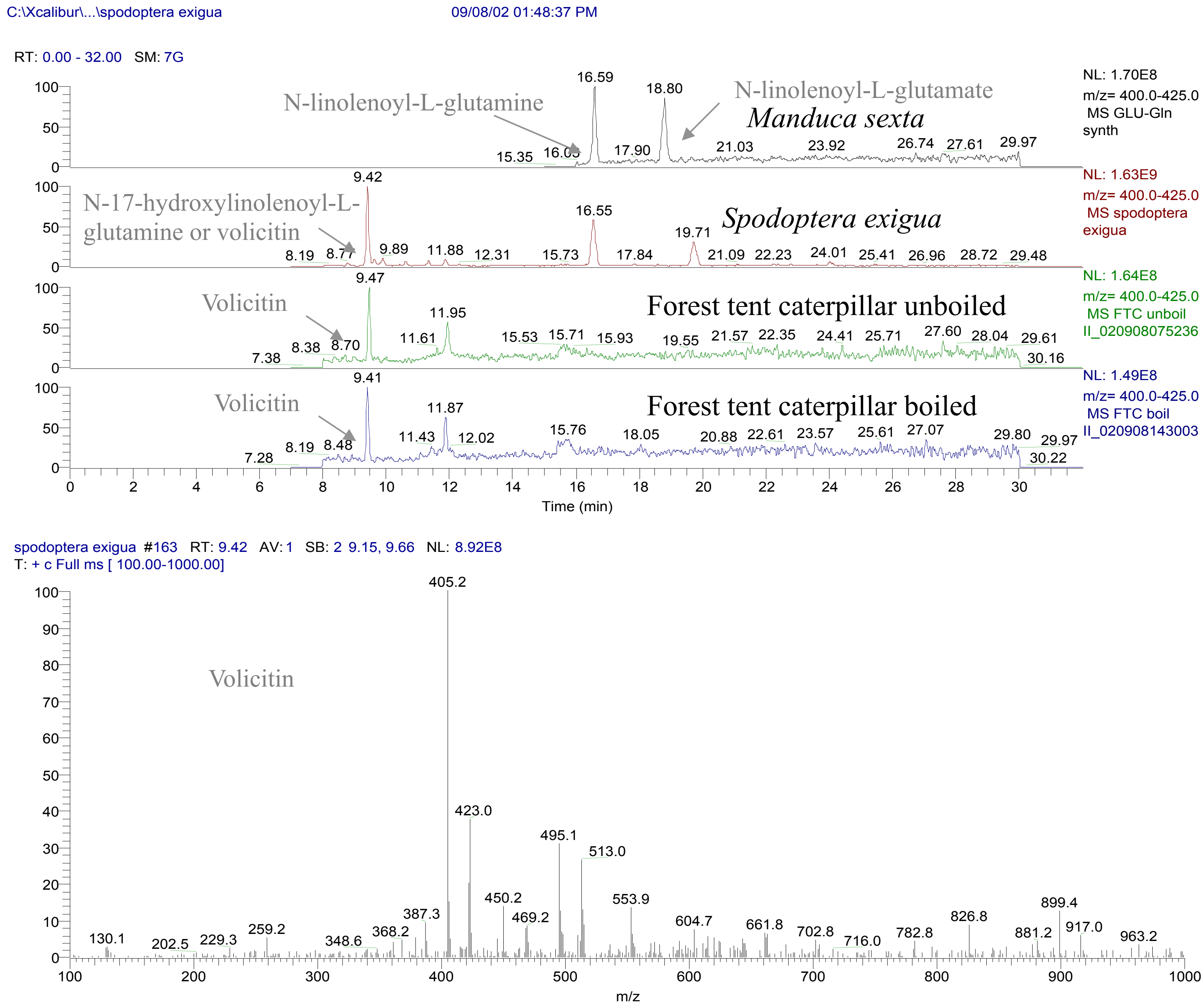
N-(17-hydroxylinolenoyl)-L-glutamine



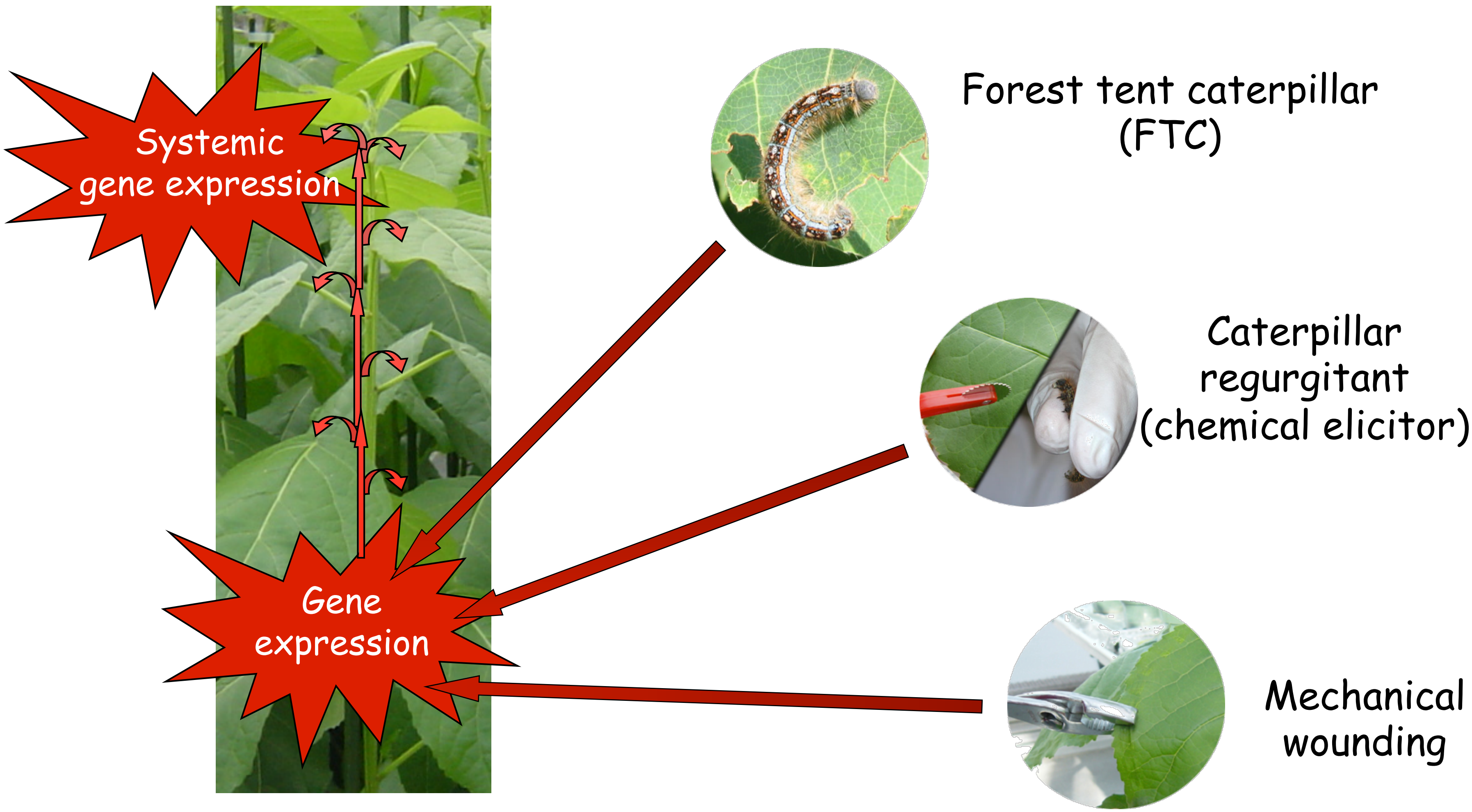
Volicitin (N-17-hydroxylinolenoyl)-L-glutamine is a jasmonate-analog

Alborn et al, Science 276: 945 (1997)

The biochemistry of caterpillar spit


C:\Xcalibur\...\spodoptera exigua
09/08/02 01:48:37 PM
RT: 0.00 - 32.00 SM: 7G
N-linolenoyl-L-glutamine
N-linolenoyl-L-glutamate
Manduca sexta
NL: 1.70E8
m/z= 400.0-425.0
MS GLU-Gln synth
N-17-hydroxylinolenoyl-L-glutamine or volicitin
Spodoptera exigua
NL: 1.63E9
m/z= 400.0-425.0
MS spodoptera exigua
Volicitin
Forest tent caterpillar unboiled
NL: 1.64E8
m/z= 400.0-425.0
MS FTC unboil II_020908075236
Volicitin
Forest tent caterpillar boiled
NL: 1.49E8
m/z= 400.0-425.0
MS FTC boil II_020908143003
Time (min)
spodoptera exigua #163 RT: 9.42 AV: 1 SB: 2 9.15, 9.66 NL: 8.92E8
T: + c Full ms [100.00-1000.00]
Volicitin
m/z

Defense of poplar against insect herbivory is an ***active process*** and can be triggered by ***multiple signals***



Macroarray analysis of the poplar herbivore defense response

What kinds of genes are induced? (*2-fold, P<0.05, 117 total*)

Putative function	accession	FTC-R rank[a]	Wound rank[a]
Poplar endochitinase *win6.2C*	CN192741	1	1
Lipase, class 3		2	5
Poplar endochitinase *win8*		6	2
Apyrase	xxxxxxxx	4	6
Poplar vegetative storage protein *wi*			
Polyphenol oxidase			
Unknown			
Kunitz trypsin inhibitor *PtdTI5*	CN192805	--[8]	7
Acid phosphatase, class B	CN193016	7	14
Pop3 / *SP1*			
Pop3- / *SP1*-like			
Acyl-activating enzyme			
Kunitz trypsin inhibitor *PtdTI3*	CN192549	17	11
Kunitz trypsin inhibitor *PtdTI4*		[26]	4
β-glucosidase			
β-amylase			
	CN193014	12	26
...de cyclase	CN193019	21	18
Cytochrome P450	CN193274	28	16
Cytochrome P450	CN193412	25	19
18S rRNA gene	CN192944	20	24
Cytochrome P450	CN193236	16	29
Unknown protein (MOSC domain)	CN193222	15	31
Phenylalanine ammonia lyase	CN192894	13	34
Cytochrome P450	CN193273	26	23
Lipolytic enzyme, G-D-S-L	CN193295	22	27
Cinnamyl alcohol dehydrogenase	CN192800	14	39
Esterase / Lipase	CN192875	--[33]	20
Unknown (DUF946 domain)	CN193384	24	37
Cytochrome P450	CN193433	27	35
Pop3- / *SP1*-like	CN192903	29	41
TB2/DP1- and HVA22-related protein	CN192744	18	52
13-Lipoxygenase	CN192531	--[49]	28
Poplar vegetative storage protein *pni288*	CN193425	--	10
Anthocyanidin synthase	CN192891	--	21
ADP-glucose pyrophosphorylase	CN192812	23	--
Neutral invertase	CN193364	--	25

Endochitinase

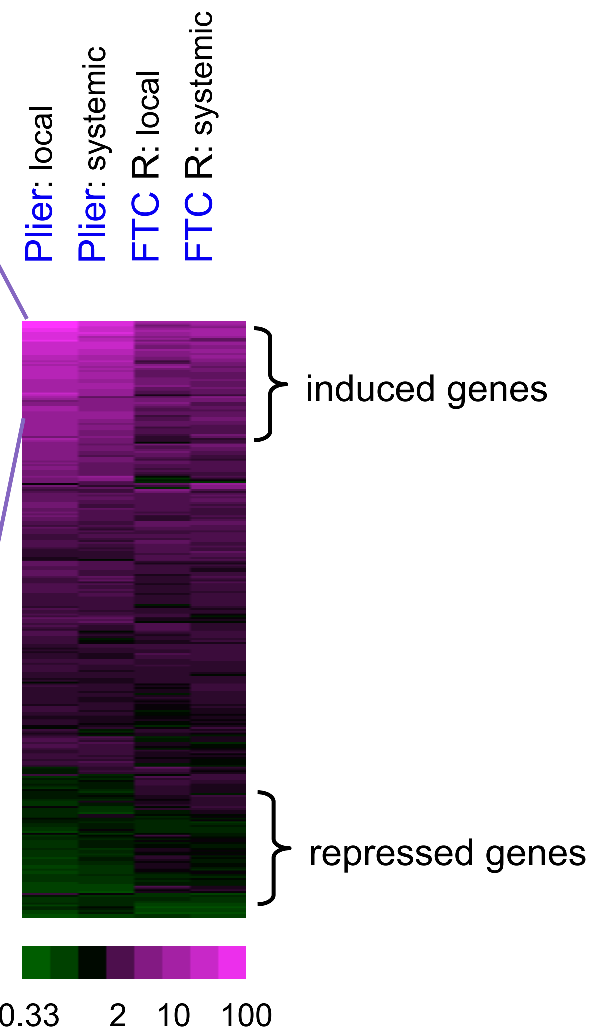
Polyphenol oxidase

Kunitz Trypsin Inhibitor-5

Kunitz Trypsin Inhibitor-3

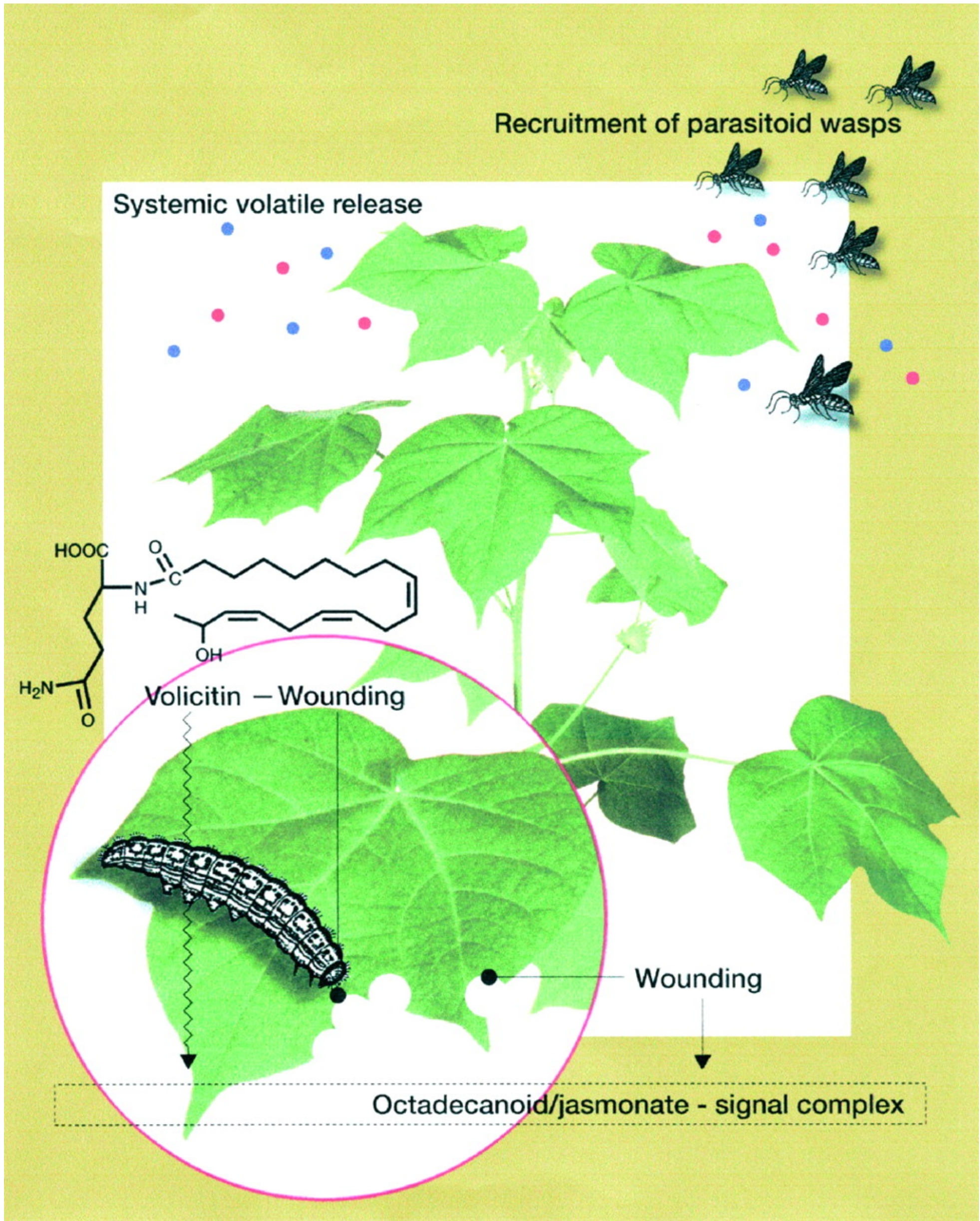
Kunitz Trypsin Inhibitor-4

Unknown - Pop3/SP1



Major and Constabel, New Phytol 172: 617-635 (2006)

Increase of volatile compounds released by plants in response to insect feeding



Par P. W., Tumlinson J. H. Plant Physiol. 1999:121:325-332