Physiological Bases of Using Insect Hormone Analogs for Pest Management

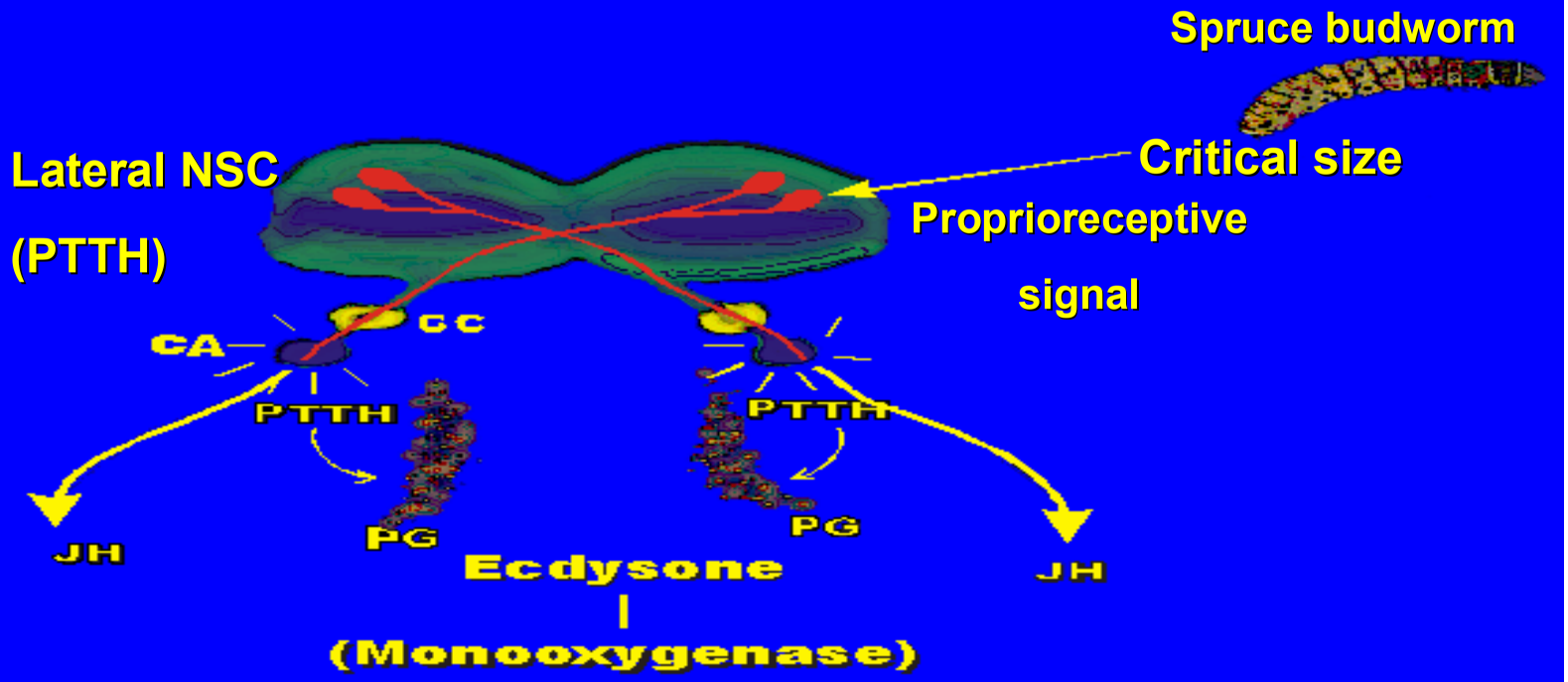
Use of tebufenozide against the spruce budworm, Choristoneura fumiferana

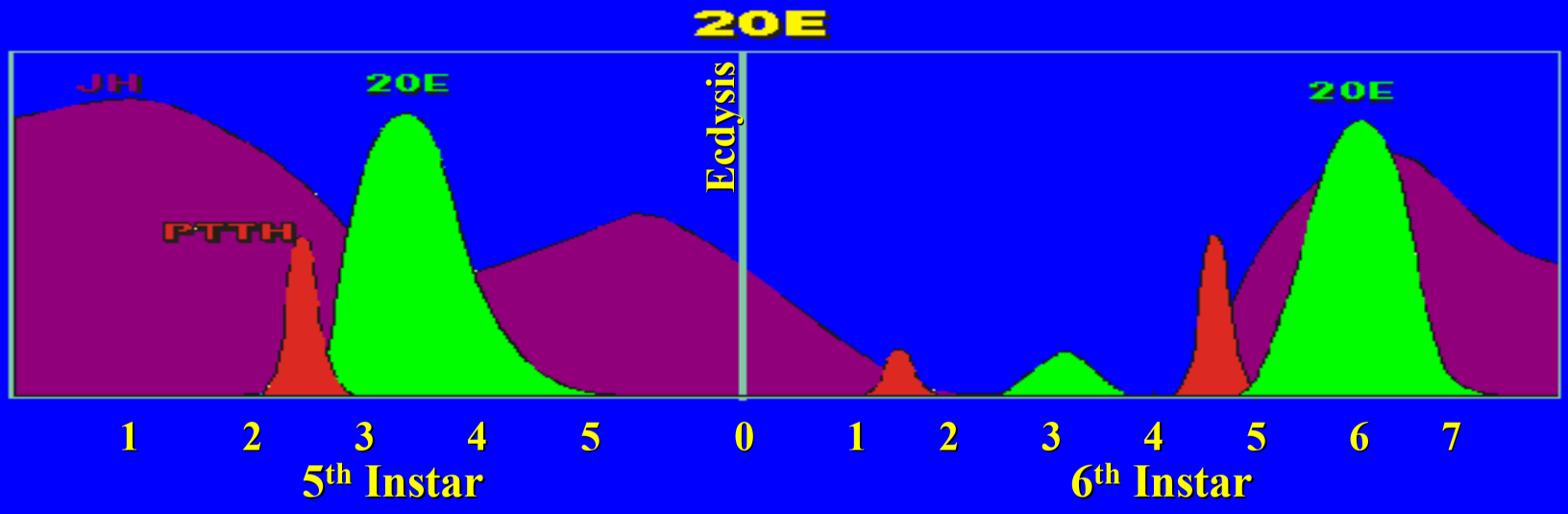
Arthur Retnakaran, Daniel Doucet, Basil M. Arif,
Great Lakes Forestry Centre, Sault Ste. Marie, Canada

Overview

- The molting cycle
- Interference of the molting cycle by diacylhydrazines
- Use of tebufenozide against the spruce budworm (*Choristoneura fumiferana*): effectiveness during diapause
- Conclusion

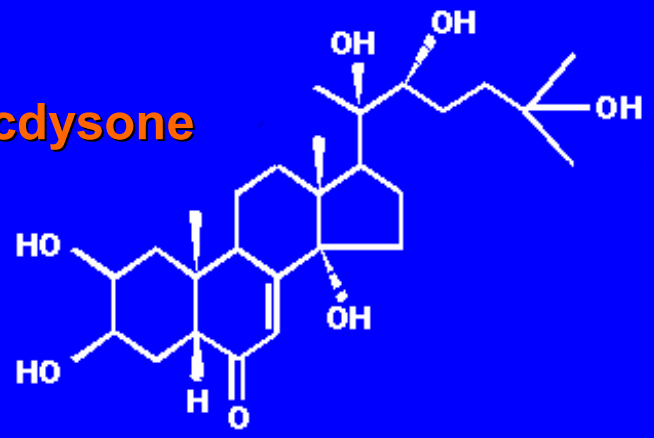
Hormonal regulation of molting and metamorphosis

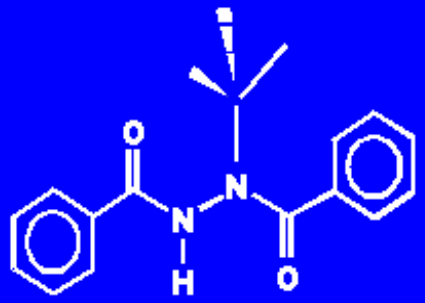




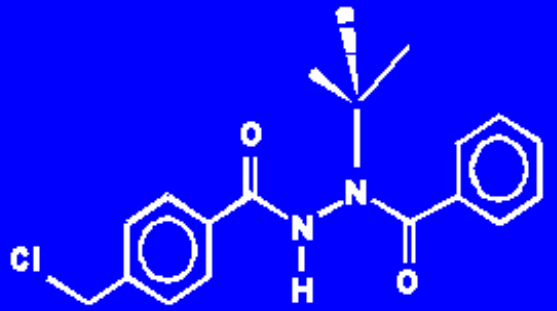
20-Hydroxyecdysone and Ecdysone Agonists

20-hydroxyecdysone

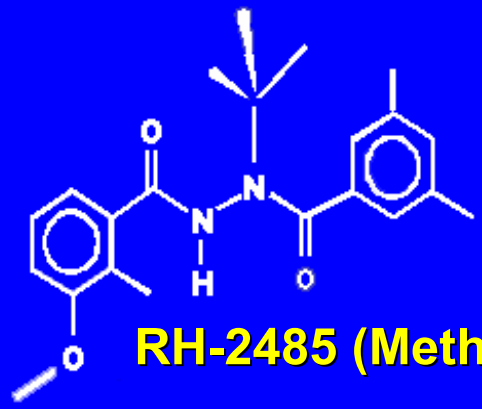




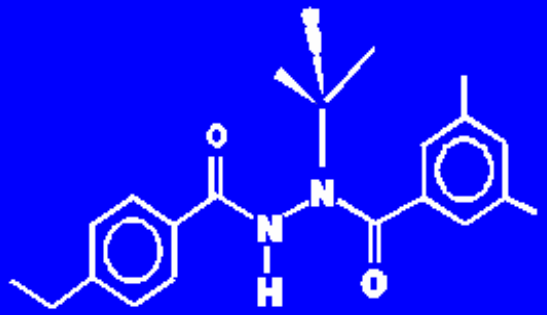
RH-5849 (original analog)



RH-0345 (Halofenozide)



RH-2485 (Methoxyfenozide)

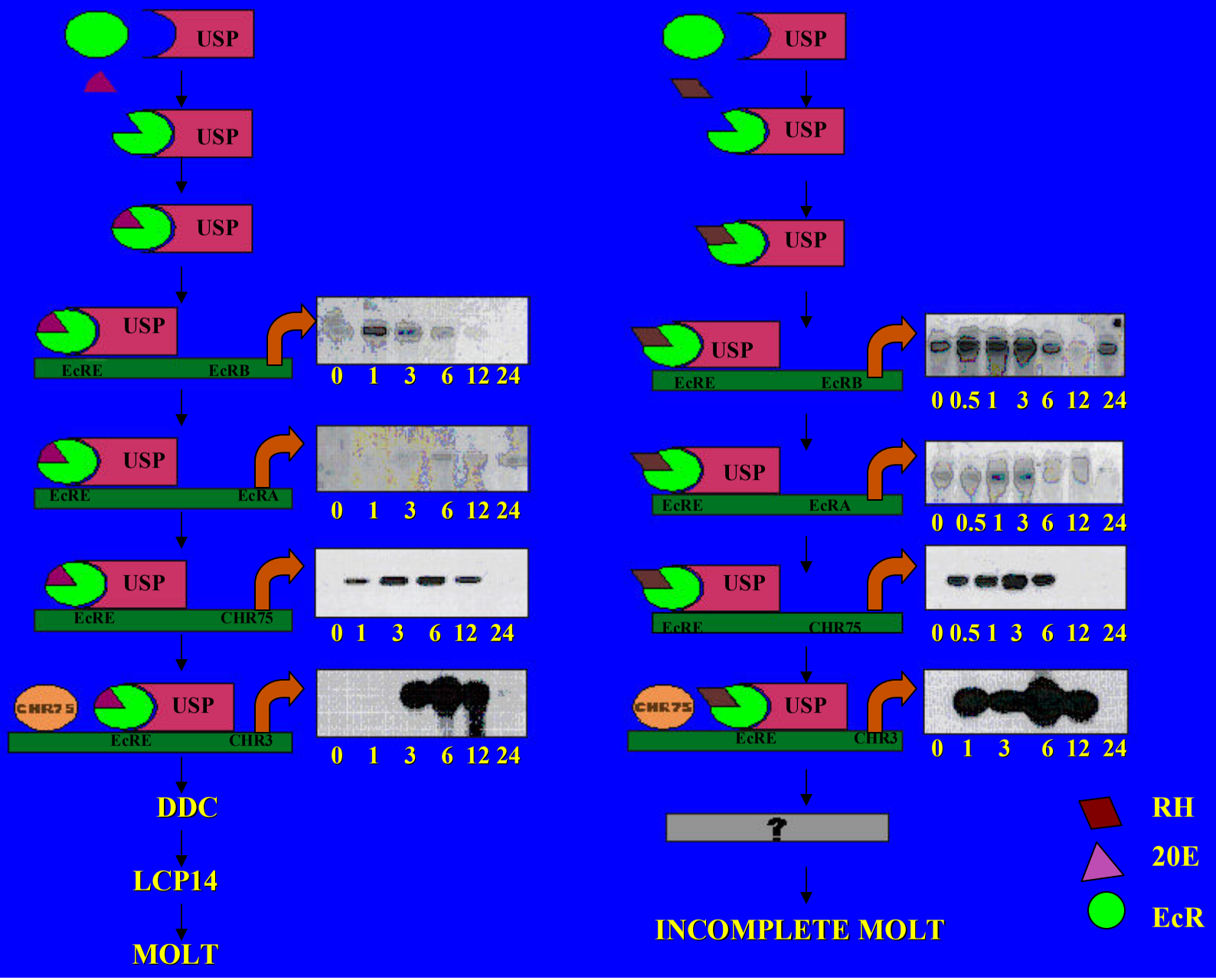


RH-5992 (Tebufenozide)

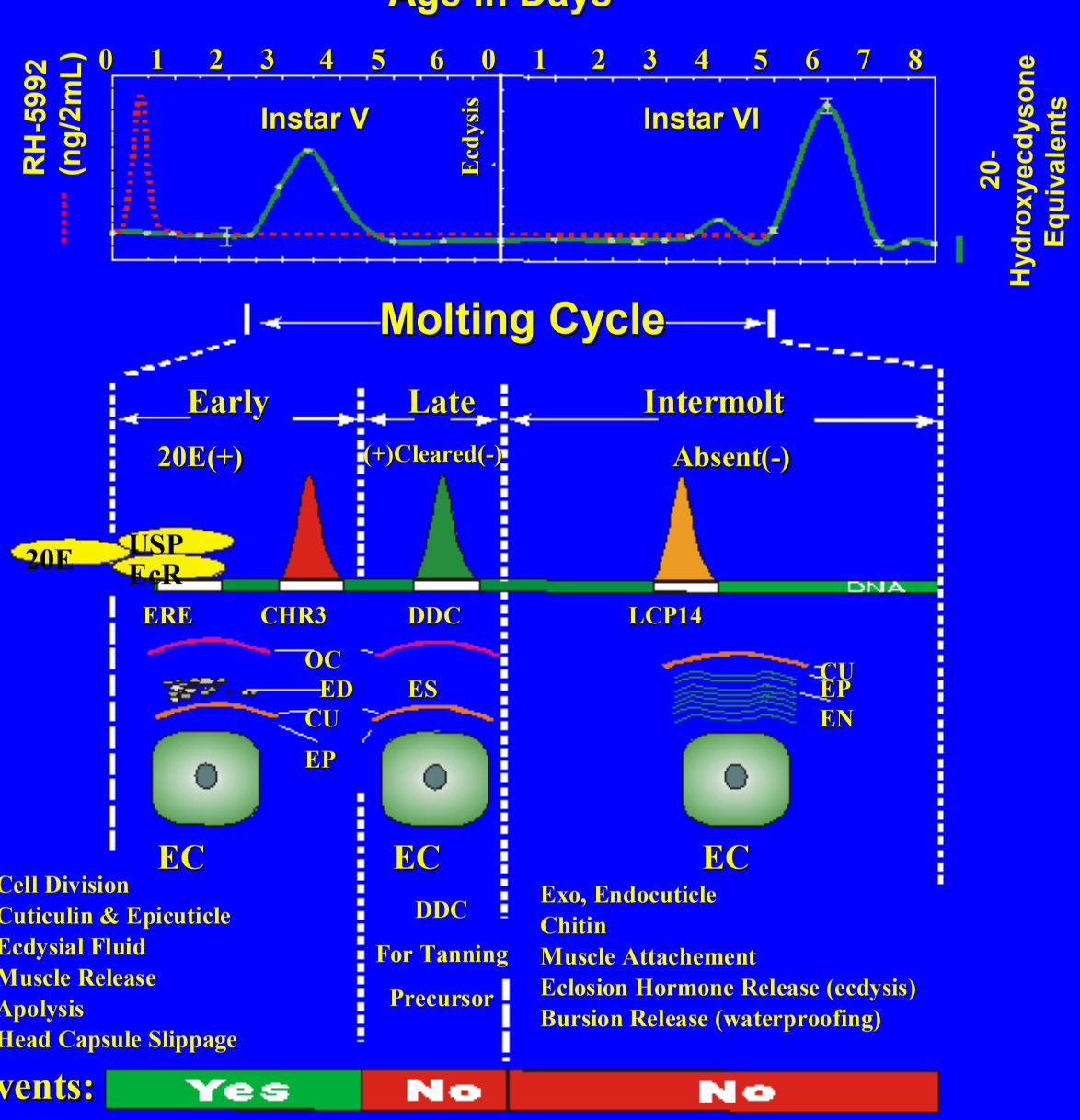
Phenotypic effects of diacylhydrazines

- Feeding inhibition within 3-14h + synthesis of new cuticle, apolysis of new cuticle from old one.
- Head capsule slippage
- New cuticle not tanned or sclerotized

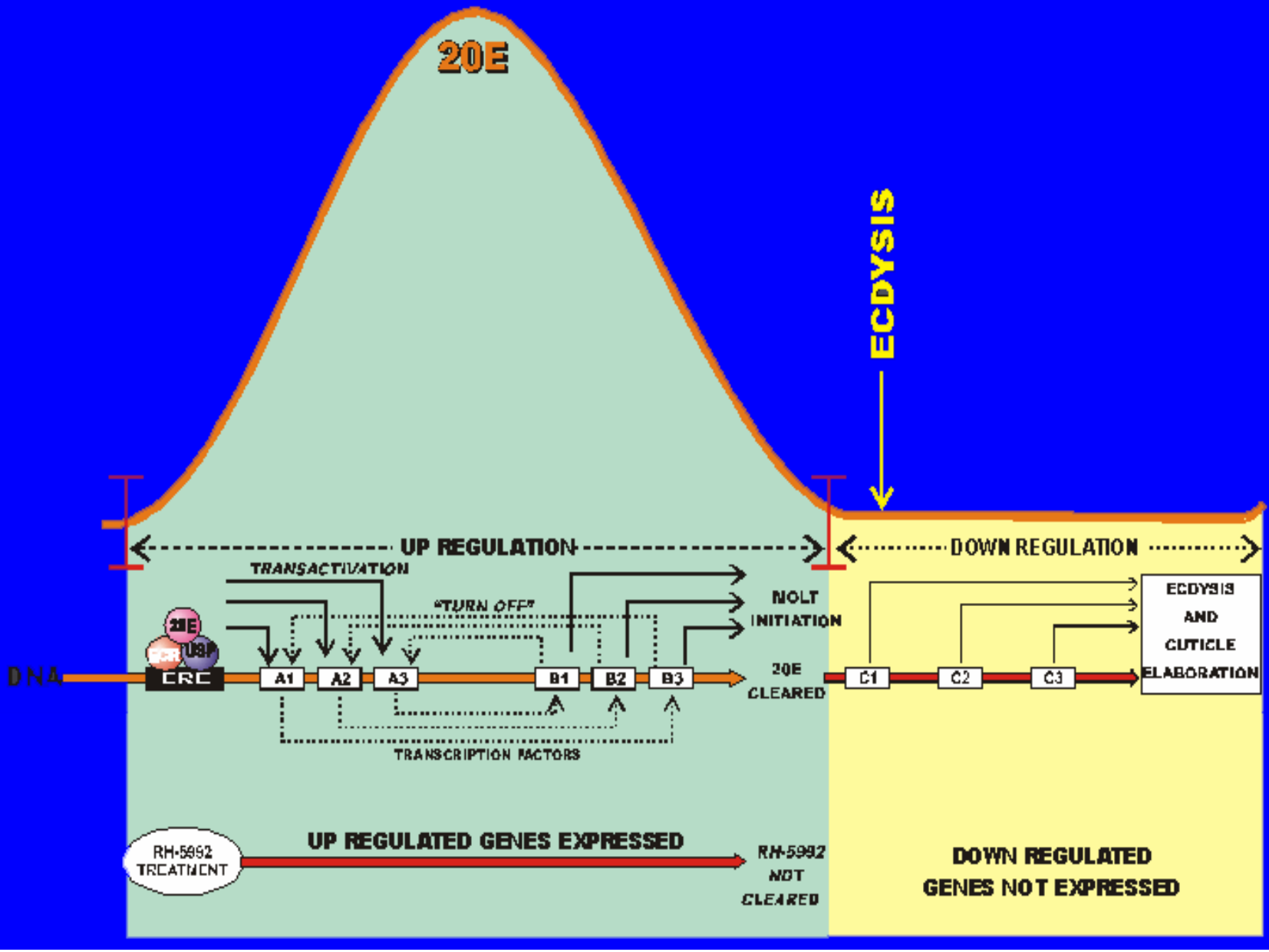
Mode of Action of RH-5992: early molecular events



Molecular Mode of Action of RH-5992



Schematic Model of the Mode of Action of Tebufenozide



Diapause

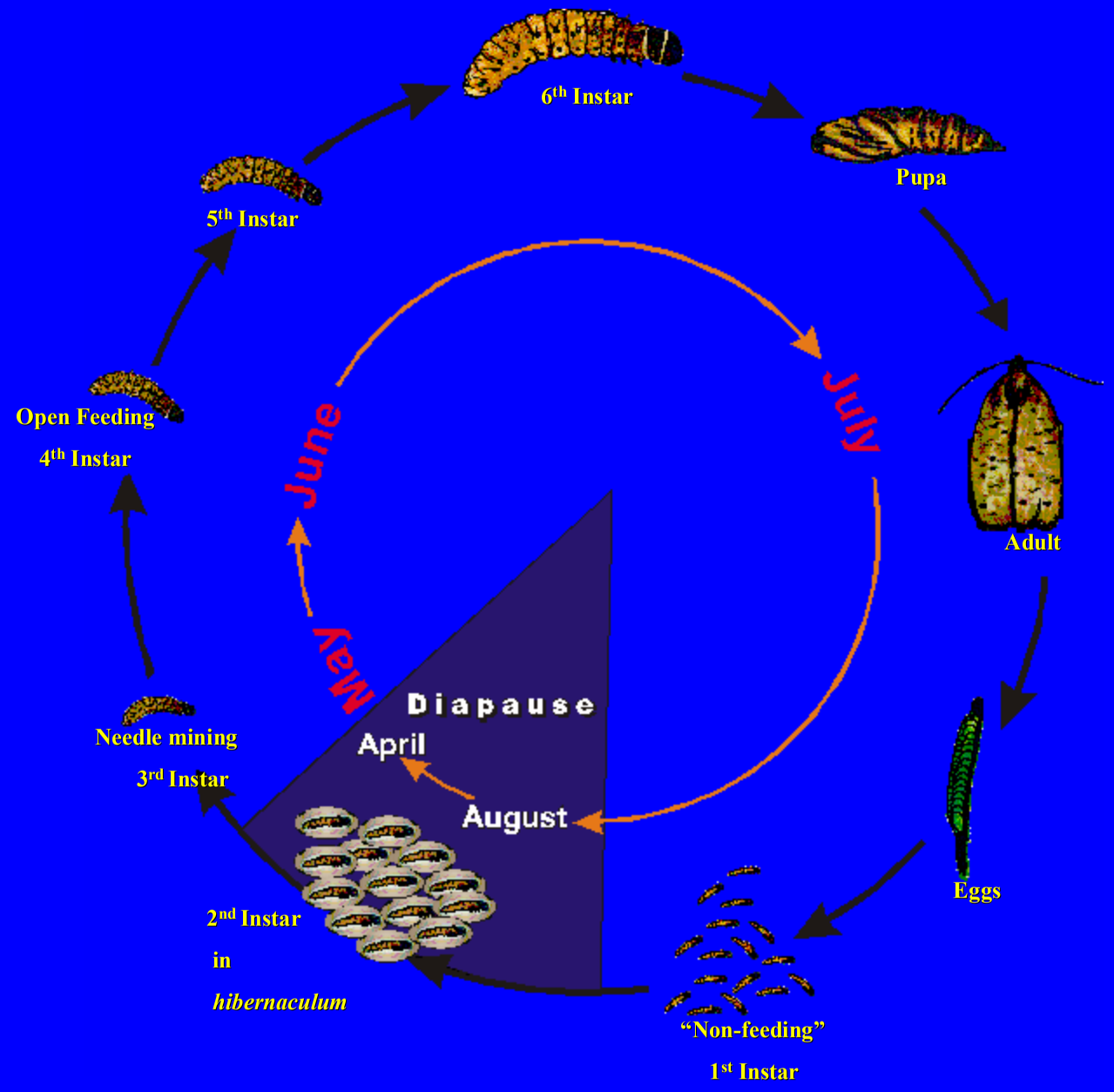
1. Developmental arrest to overcome seasonal adversity.
2. Obligatory or facultative. Embryonic, Larval Pupal or Adult.
3. Onset & termination controlled by temp and or photoperiodism.
4. Photoperiodic clock regulated by several genes, period and timeless accumulate during scotophase.
5. Induction ahead of diapause for preparation - storage proteins, glycerol, sorbitol.
6. Types of diapause:
   a) Embryonic - Diapause hormone - Silk worm
   b) Larval, Pupal - Shut down Brain (PTTH), Prothoracic gland (20E)
   c) Adult - no JH

Diapause in Insects

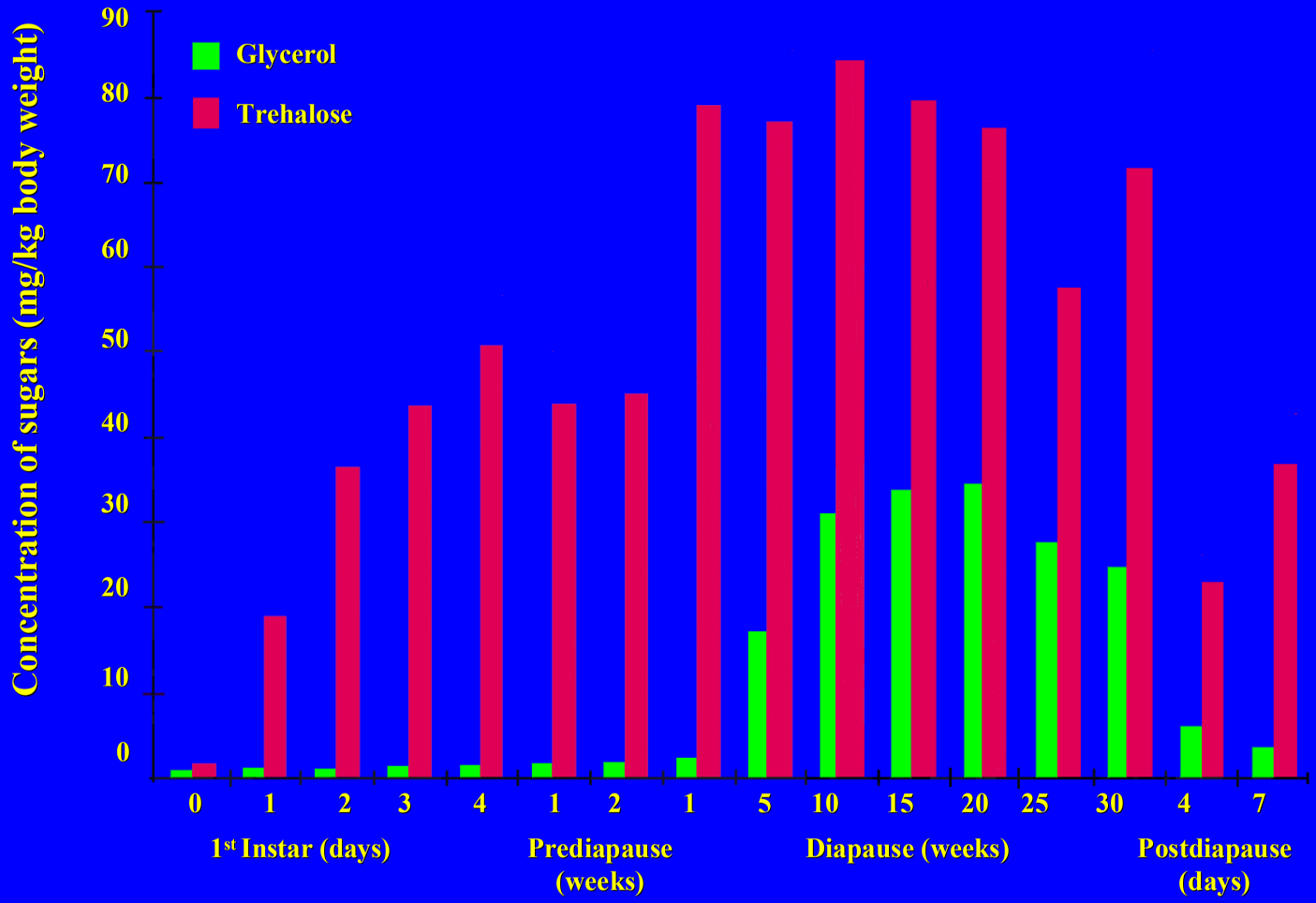
Stage	Environmental Signal	Endocrine Regulation	Example
Egg (embryonic)	LD + High Temp	Diapause Hormone	Silkworm
Early Larval	SD ± Low Temp	No 20E + JH(?)	Spruce Budworm
Last Larval	SD ± Low Temp	No 20E – JH	Corn borer
Pupal	SD ± Low Temp	No 20E	Flesh Fly
Adult	SD ± Low Temp	No JH	White Pine Weevil

Life cycle of the spruce budworm

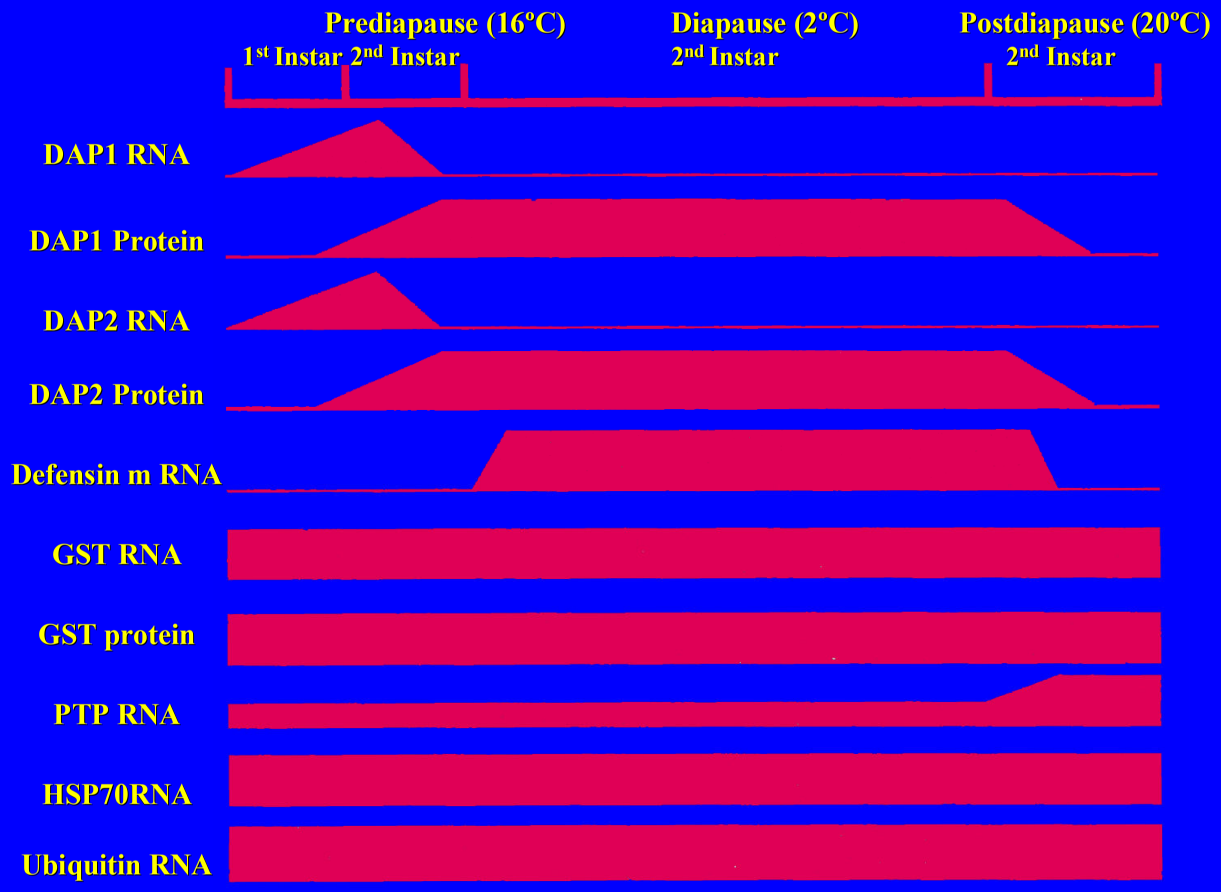
Choristoneura fumiferana

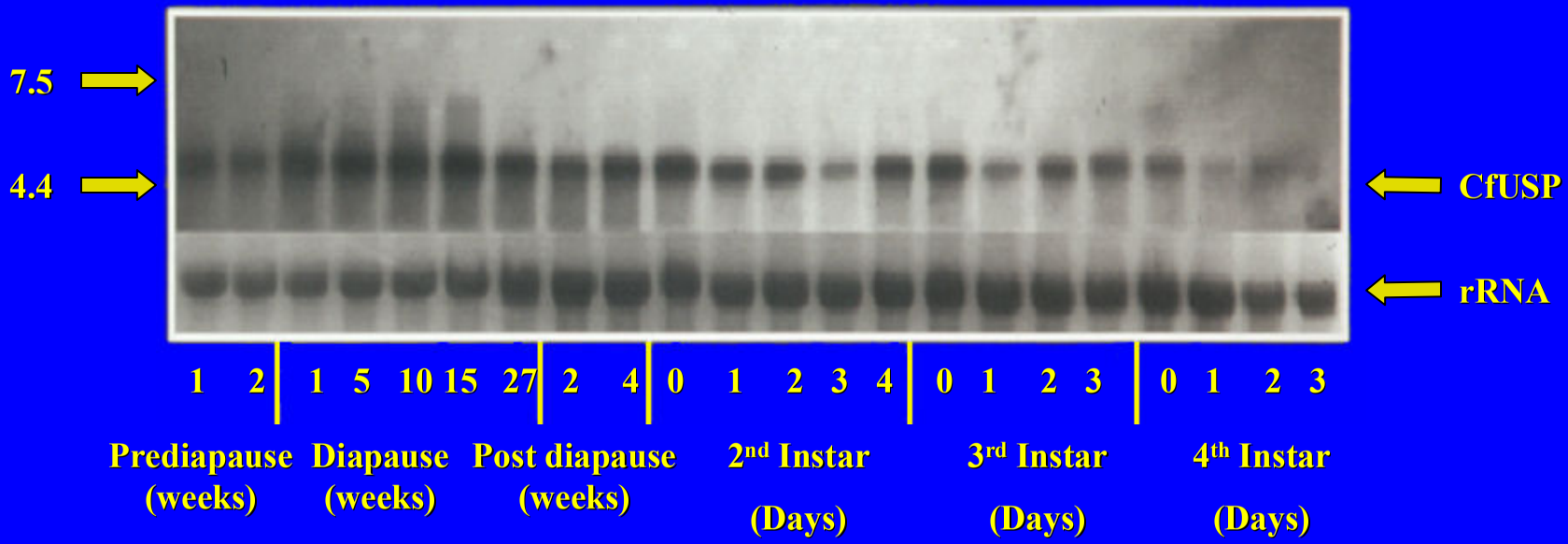


Glycerol and Trehalose Levels in First and Second Instars

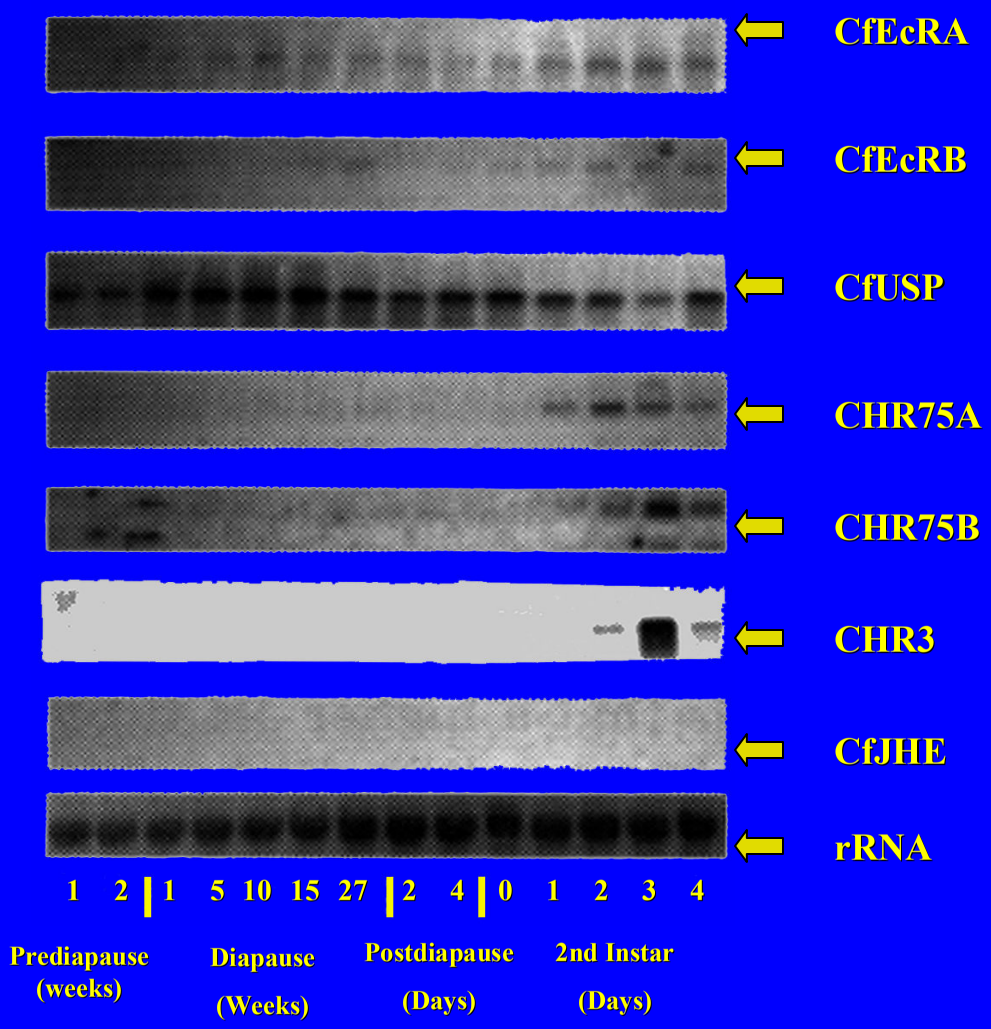


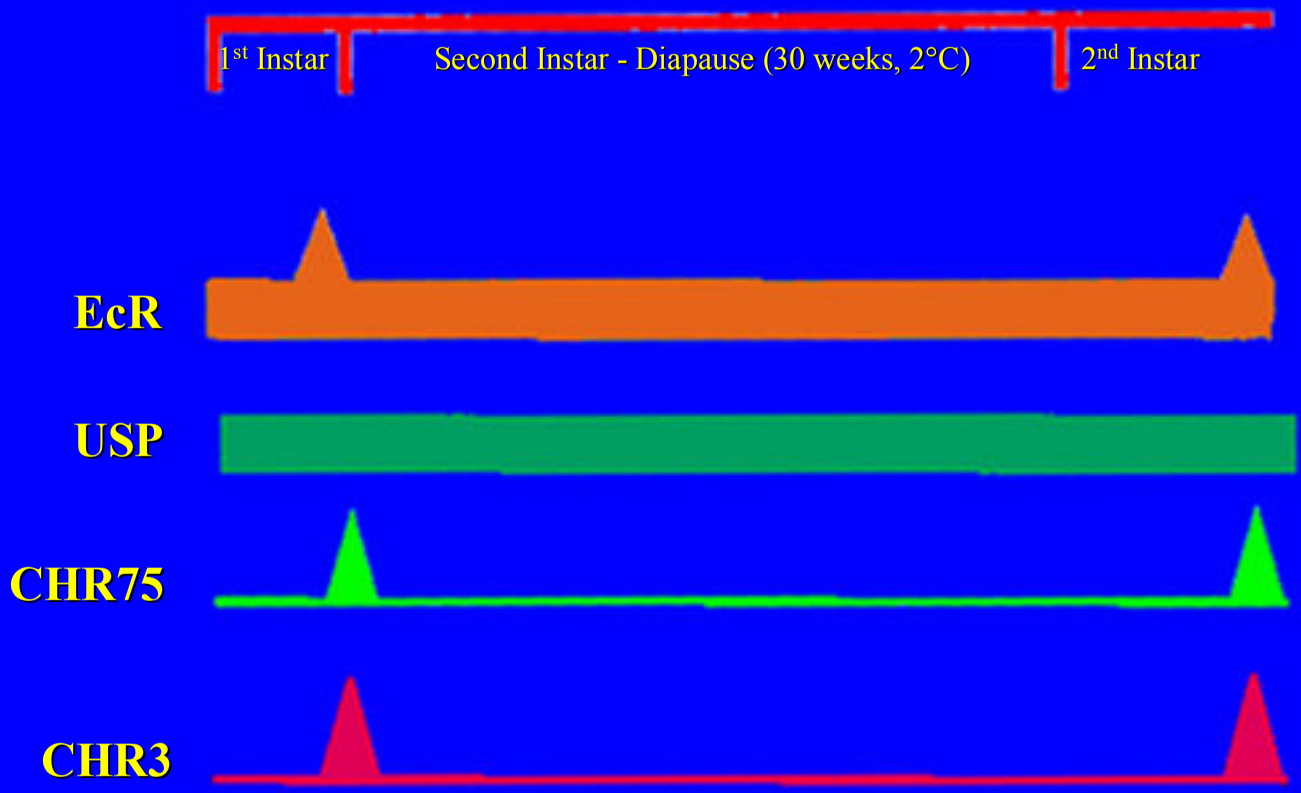
Expression Profiles of Some mRNAs and Proteins During Prediapause, Diapause and Postdiapause of First and Second Instars




7.5
4.4
CfUSP
rRNA
1 2 1 5 10 15 27 2 4 0 1 2 3 4 0 1 2 3 0 1 2 3
Prediapause (weeks)
Diapause (weeks)
Post diapause (weeks)
2nd Instar (Days)
3rd Instar (Days)
4th Instar (Days)

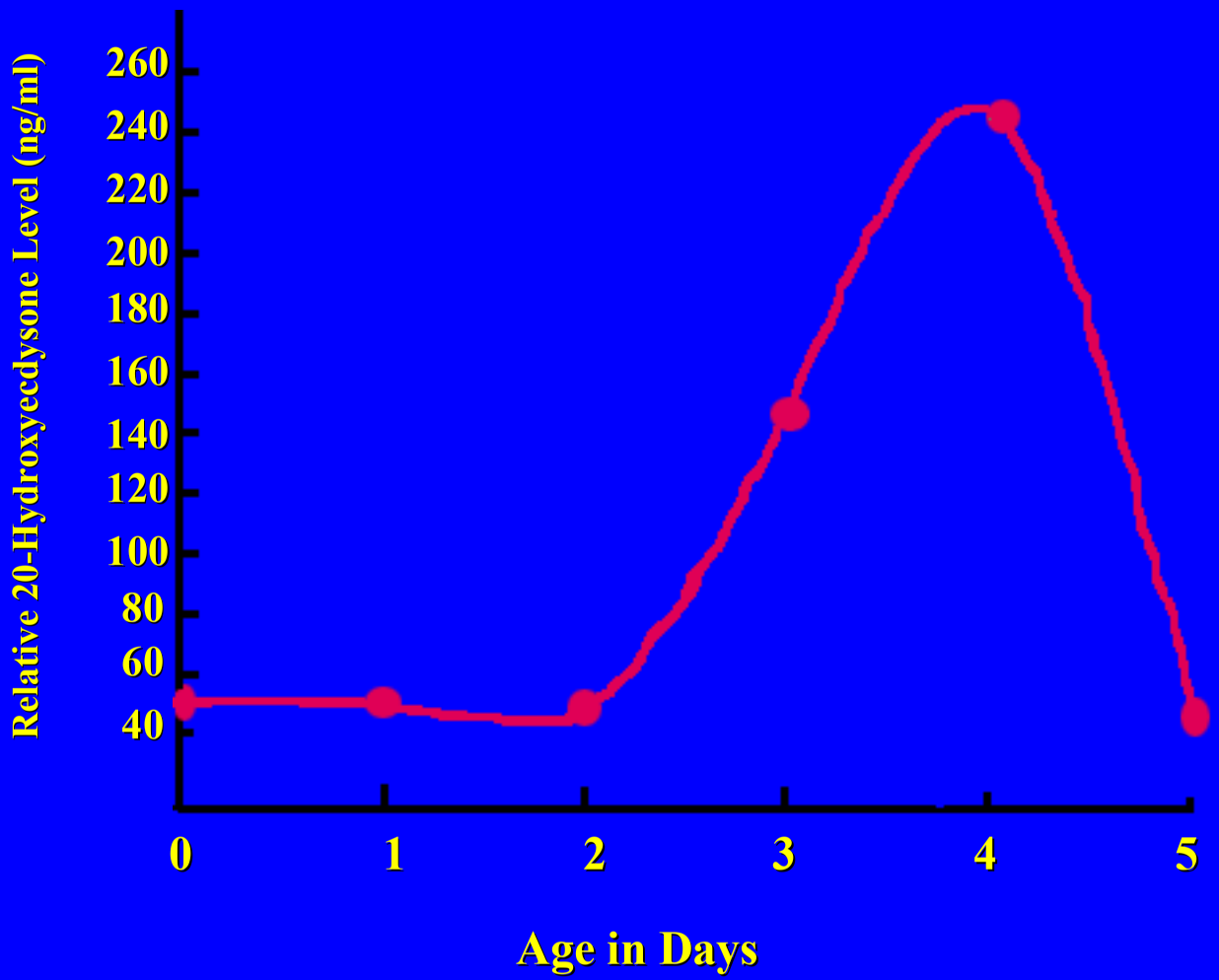
Some Ecdysone Related mRNA Expression Profiles of *Choristoneura Fumiferana*

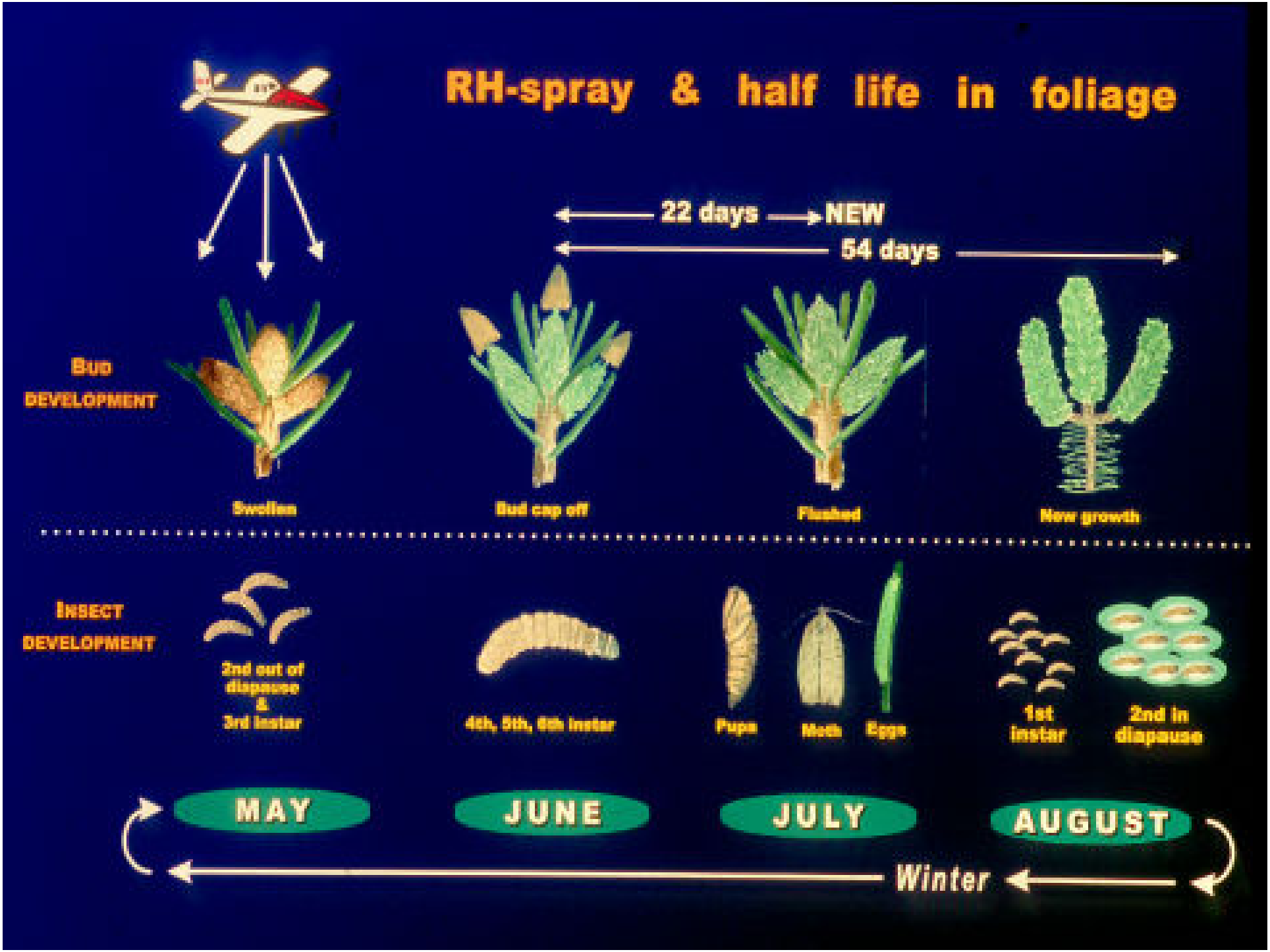



1st Instar
Second Instar - Diapause (30 weeks, 2°C)
2nd Instar
EcR
USP
CHR75
CHR3

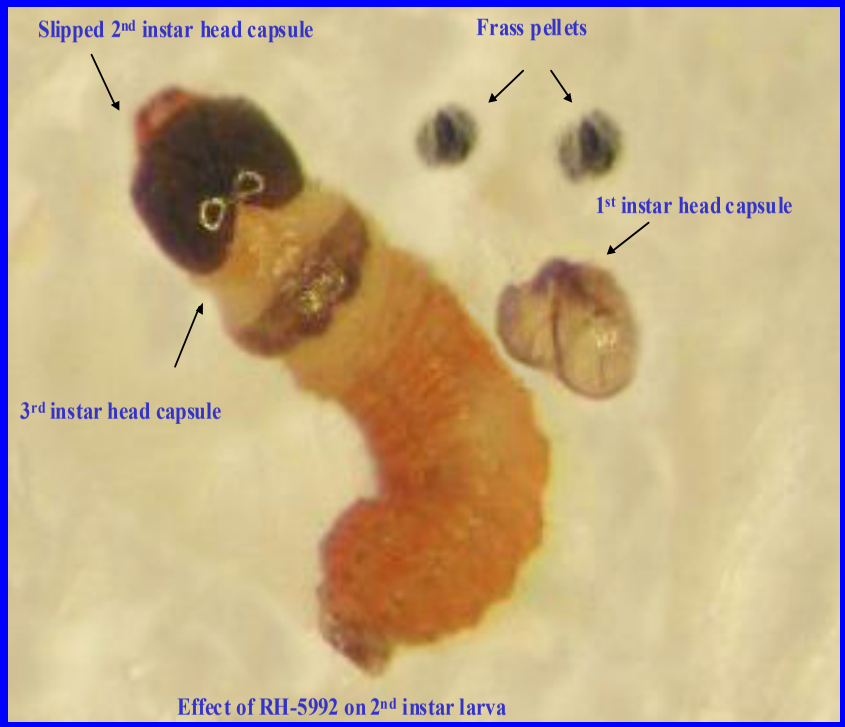
20-Hydroxyecdysone Profile

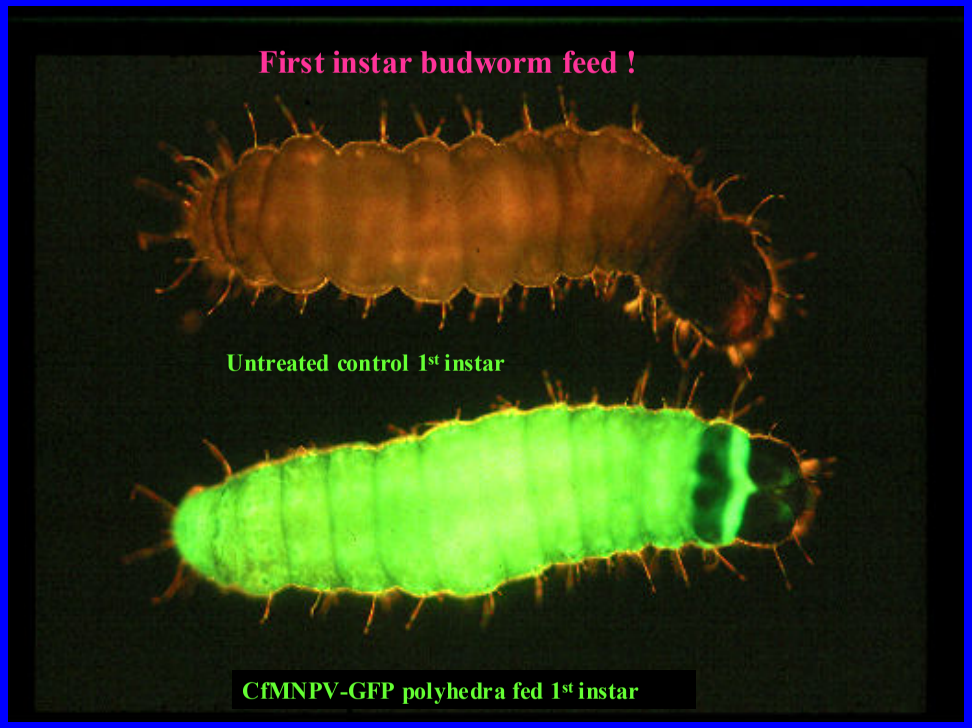
1st Instar Spruce Budworm

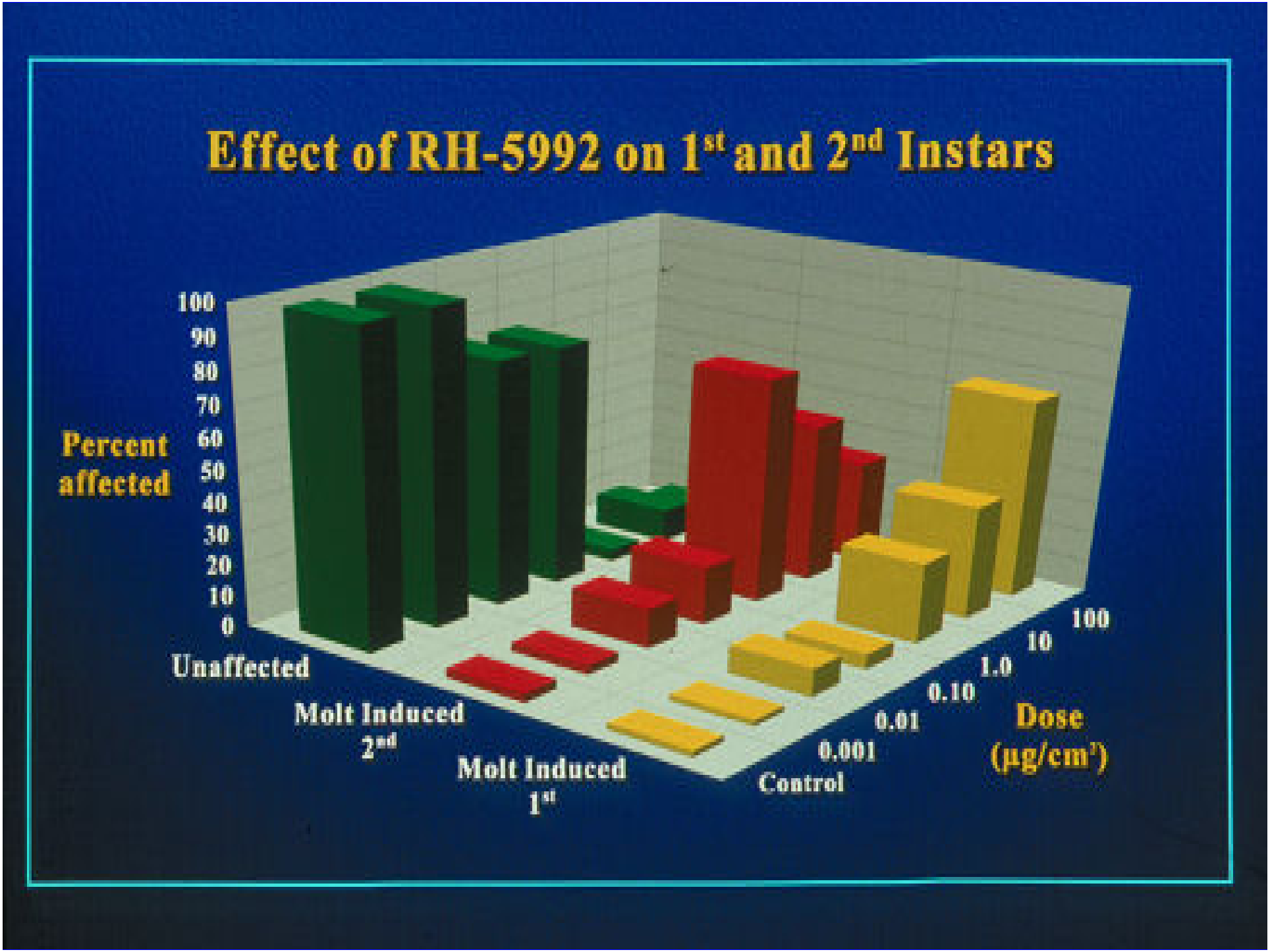



RH-spray & half life in foliage
22 days
NEW
54 days
BUD DEVELOPMENT
Swollen
Bud cap off
Flushed
New growth
INSECT DEVELOPMENT
2nd out of diapause & 3rd instar
4th, 5th, 6th instar
Pupa
Moth
Eggs
1st instar
2nd in diapause
MAY
JUNE
JULY
AUGUST
Winter

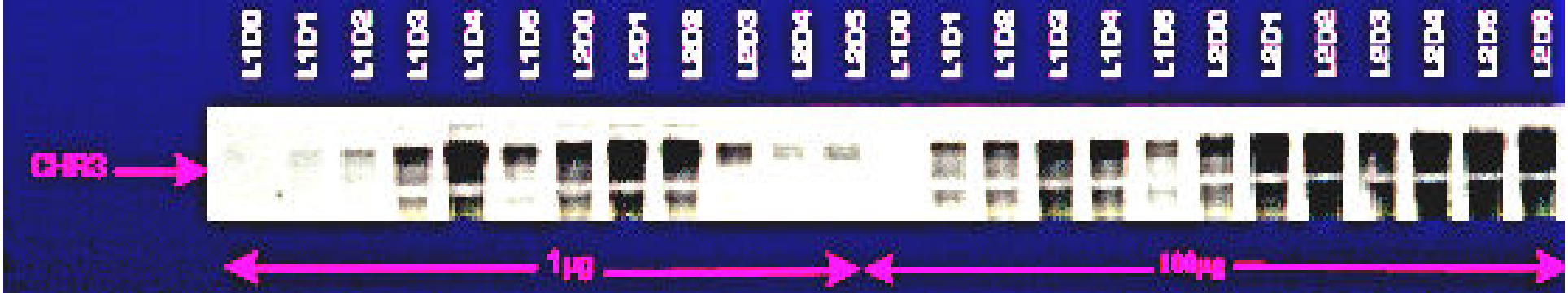

2 frass pellets
1st instar head capsule
Normal 2nd instar inside hibernaculum.

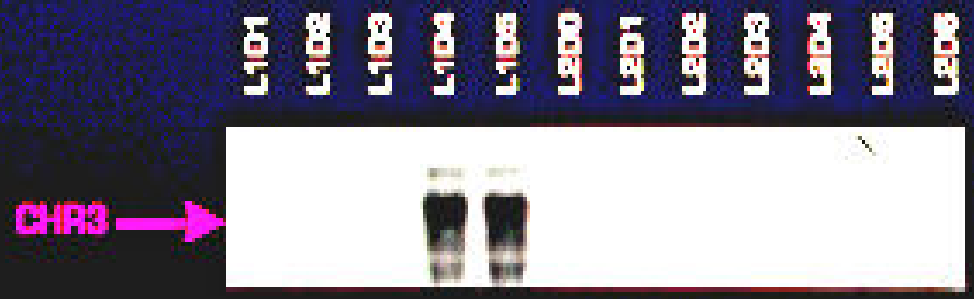

Slipped 2nd instar head capsule
Frass pellets
1st instar head capsule
3rd instar head capsule
Effect of RH-5992 on 2nd instar larva


First instar budworm feed !
Untreated control 1st instar
CfMNPV-GFP polyhedra fed 1st instar

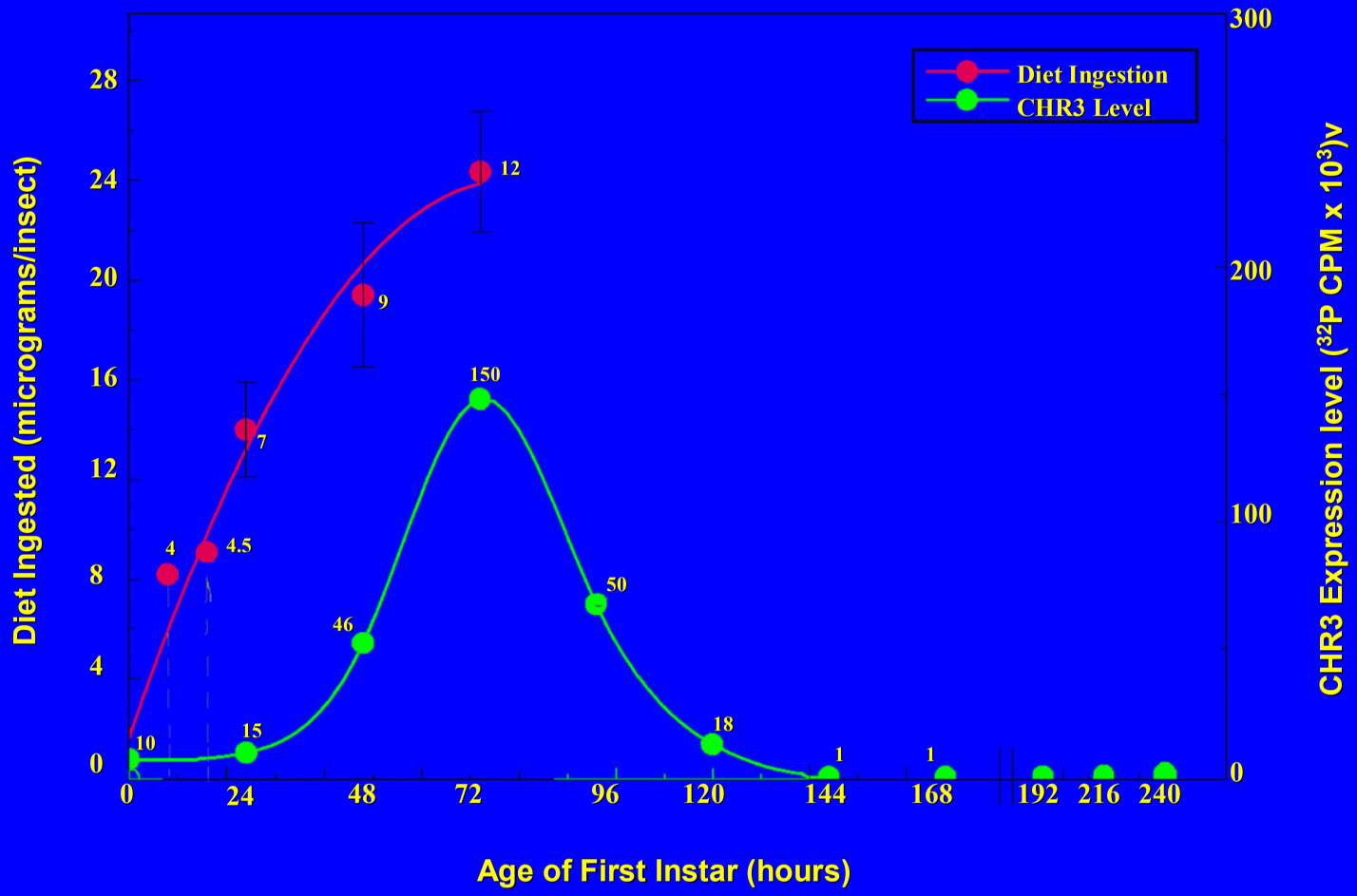

Effect of RH-5992 on 1st and 2nd Instars
Percent affected
100
90
80
70
60
50
40
30
20
10
0
Unaffected
Molt Induced 2nd
Molt Induced 1st
Control
0.001
0.01
0.10
1.0
10
100
Dose (µg/cm²)

CHR3 Induction by RH-5992 in 1st and 2nd Instar Spruce Budworm





Ingestion of Diet and CHR3 Expression in First Instar Spruce Budworm



Summary

1. RH-5992 binds strongly, is not cleared, has to be ingested before 20E peak and is carried over to the next instar. The precocious molt induced is incomplete because the down-regulated events do not occur.

- The spruce budworm is univoltine, has 6 larval instars and diapauses as a 2nd instar for 8 months.

3. Glycerol and trehalose are high; DAP 1 and 2, Defensin and GST are highly expressed but JHE peak is conspicuously absent. USP and EcR are present making the 2nds responsive to 20E.

4. The effect of RH-5992 on diapausing 2nds was discovered serendipitously during spray trials. First instars are less sensitive because they consume critical doses after the 20E peak.