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Topic A: New Discoveries
Sub-Topic A-4: Natural Product

Bioorganic Chemistry on Sex Pheromones Secreted by Lepidopteran Insects and Their Application for Plant Protection

Tetsu Ando

Graduate School of Bio-Applications and Systems Engineering (BASE)
Tokyo University of Agriculture and Technology

Introduction ①

Pheromone ← **pherein + hormon**

"Pheromone" should be designated substances that ate secreted by an animal to the outside and cause a specific reaction in a receiving individual of the same species, e.g. a release of certain behavior or a determination of physiologic development. Karlson *et al*., *Nature*, **183**: 55 (1959)

Insect pheromones: Aggregation pheromone, Alarm pheromone, Trail pheromone, Sex pheromone *etc.*

Lepidopteran sex pheromone:

Production by ♀ to attract ♂

Main factor for reproductive isolation

⇒ Species specific

Lepidoptera: ca. 150,000 species

⇒ Diversity of chemical structure

Blending of multiple components

Introduction ②

Lepidopterous sex pheromones: 500 species

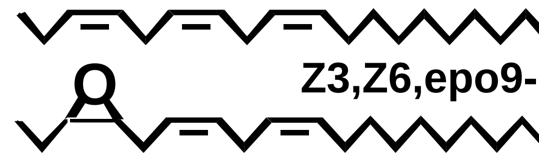
Sex attractants: 1200 species

Tipe I Unsaturated fatty alcohols, acetates, and aldehydes derivatives (C_{10}-C_{18})

silkworm moth

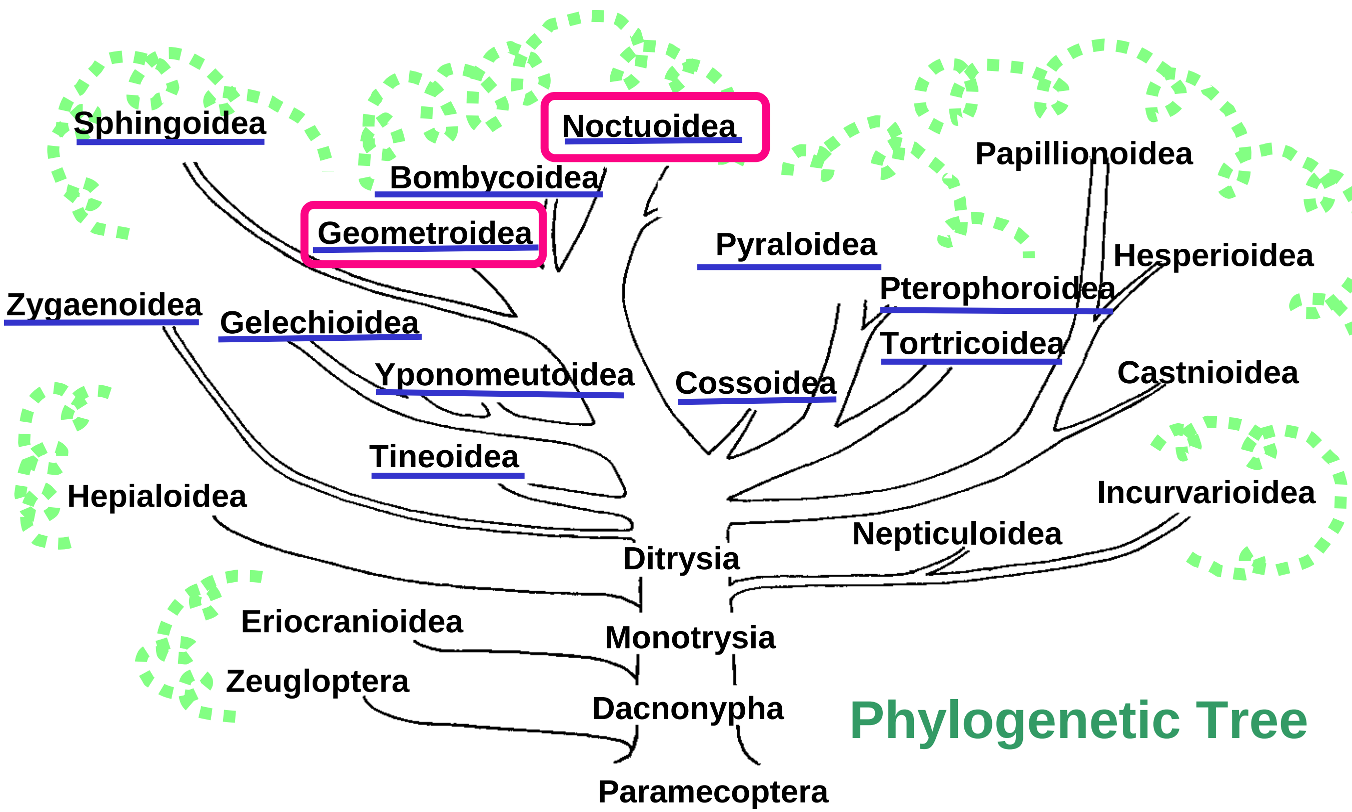
E10,Z12-16:OH (bombykol)

Tipe II (3*Z*,6*Z*,9*Z*)-trienes, (6*Z*,9*Z*)-dienes, and their monoepoxides (C_{17}-C_{23})



Z3,Z6,epo9-18:H

(10:1) Z6,epo9-18:H



Phylogenetic Tree

Introduction ③

Pheromone Studies by Chemical Ecology Laboratory in TUAT

(1) Systematic synthesis and field evaluations

Finding of new attractants for male moths by random screening

Preparation of authentic standards for identification

(2) Identification of natural pheromones

Development of new analytical techniques

Understanding of mechanisms of reproductive isolation

(3) Application for pest control

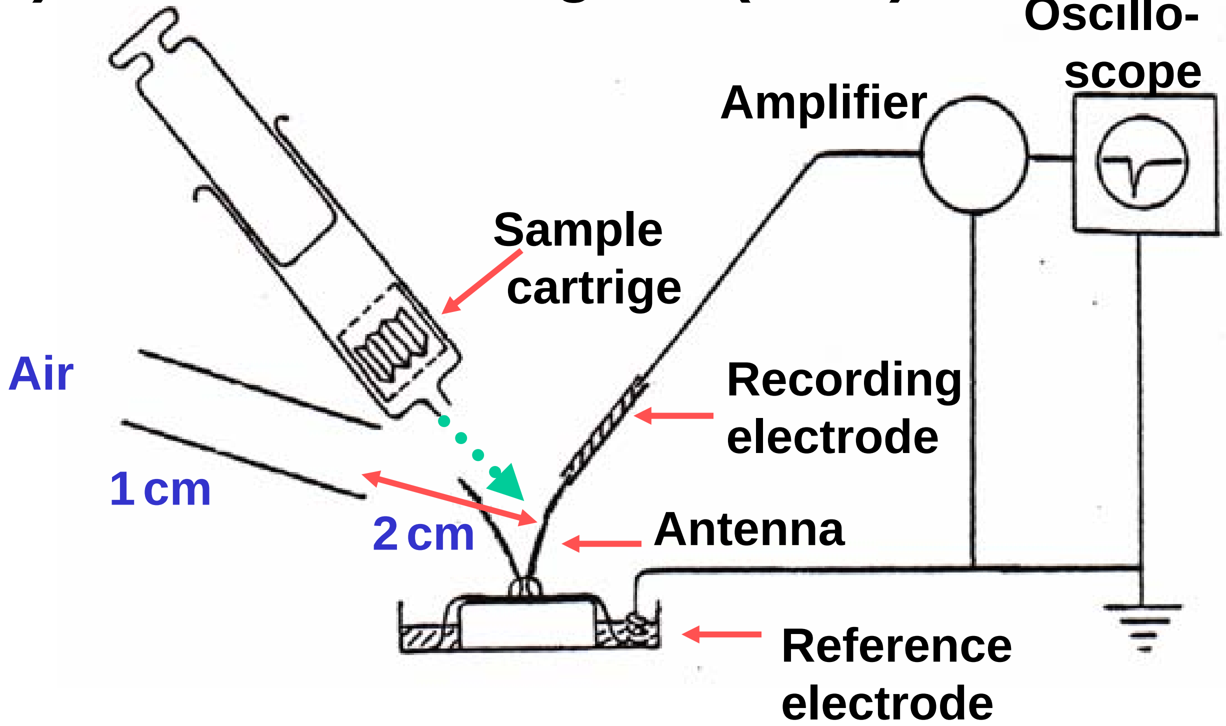
Establishment of new monitoring and disruption tools

(4) Biosynthesis and olfactory perception

Experimental confirmation of biosynthetic pathway, substrate specificity of enzymes, and endocrine control system

I. EAG Technique ①

(A) Electroantennogram (EAG)



Schneider, 1969
Science, **163**: 1031
First measurement

Roelofs *et al*., 1971
Science, **174**: 297
First application as
a bioassay method

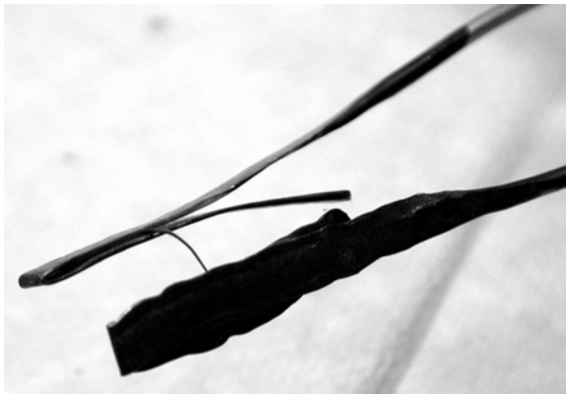
Arn *et al*., 1975
Z. Naturforsh,
30c: 722
GC-EAD

Merits： Multiple measurements are possible by one antenna
in a bright room without any conditioning of the male.
The antenna make a response to each separated component.

Demerits： Certification for the field attraction is lack.
Chemicals related to a real pheromone also show some activity.

I. EAG Technique ②

(B) GC-EAD instrument

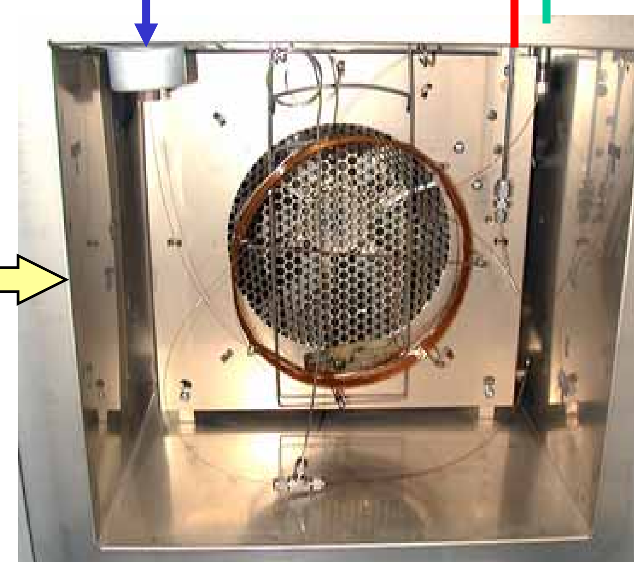


electrodes

injection

FID

EAD

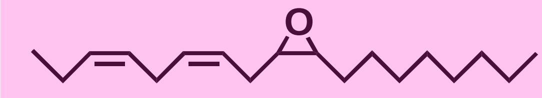


I. EAG Technique ③

(C) GC-EAD measurements of the type II pheromone

mulberry looper

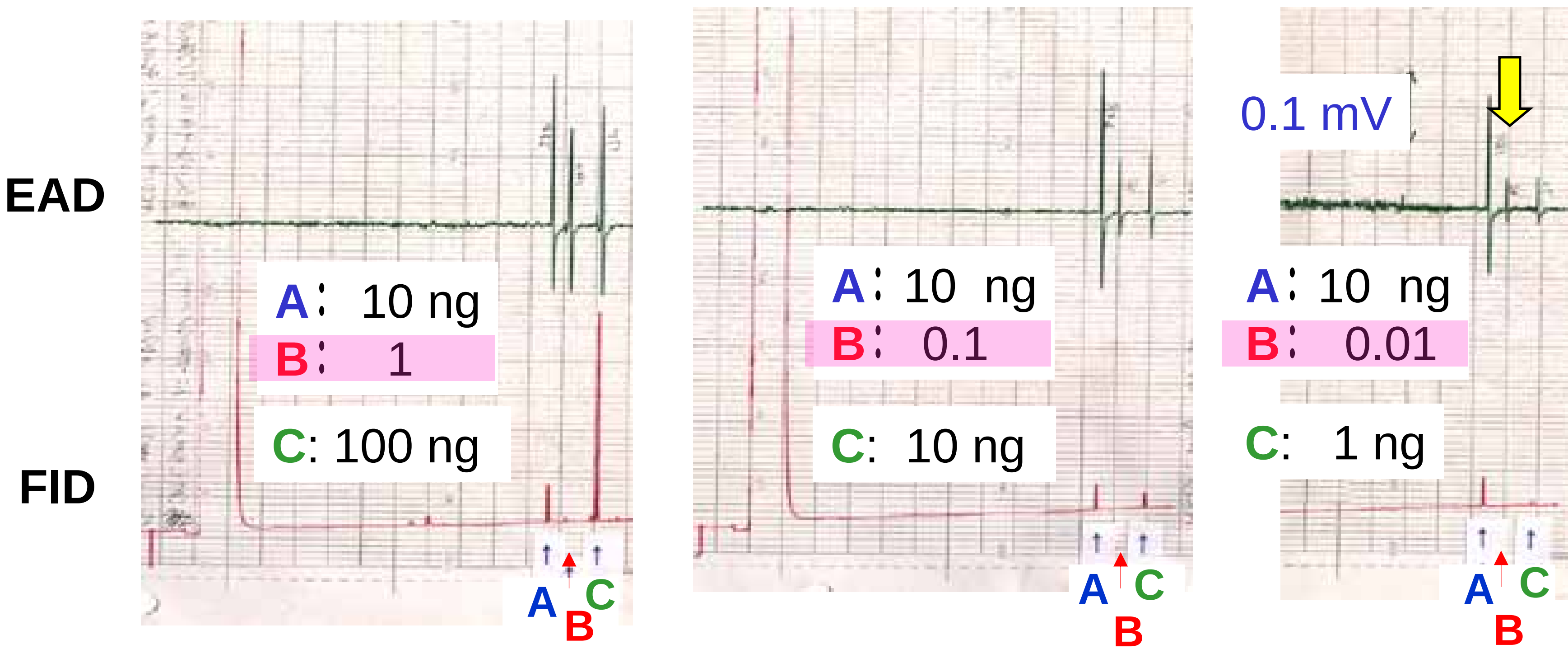
Hemerophila artilineata



Comp. **A**: Z6,epo9-18:H (minor comp.)

Comp. **B**: Z3,Z6,epo9-18:H (major comp.) ⇒ Threshold < 0.01 ng

Comp. **C**: Z3,Z6,epo9-19:H



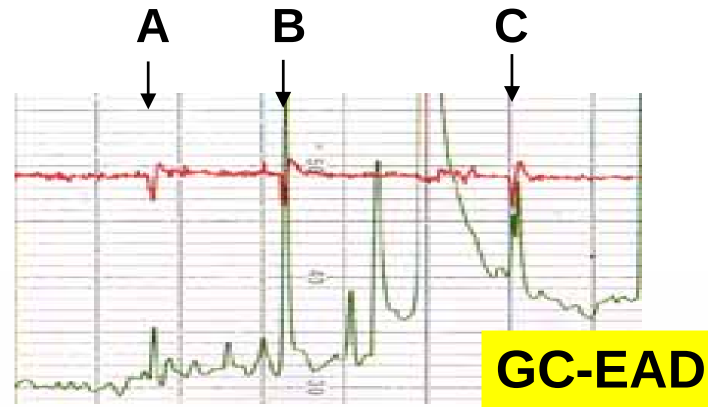
Capillary GC: good separation
EAD: high sensitivity

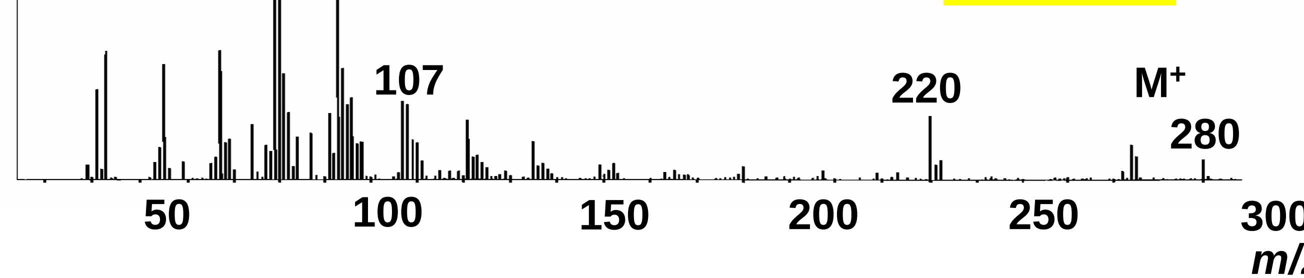
⇒ One of the most useful instruments for pheromone research

II. GC-MS Analysis ①

(A) Type I pheromone of the persimmon fruit moth [1]

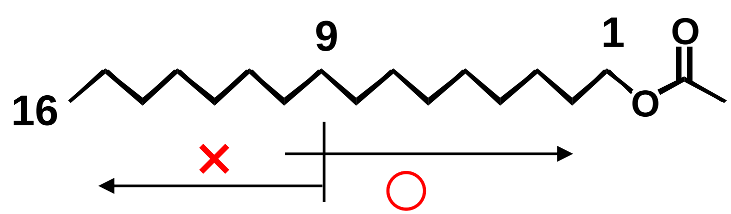
Stathmopoda masinissa
(Oecophoridae)



$C_{16}H_{29}$-O-$COCH_3$ $\Delta = 2+1$

conjugated diene



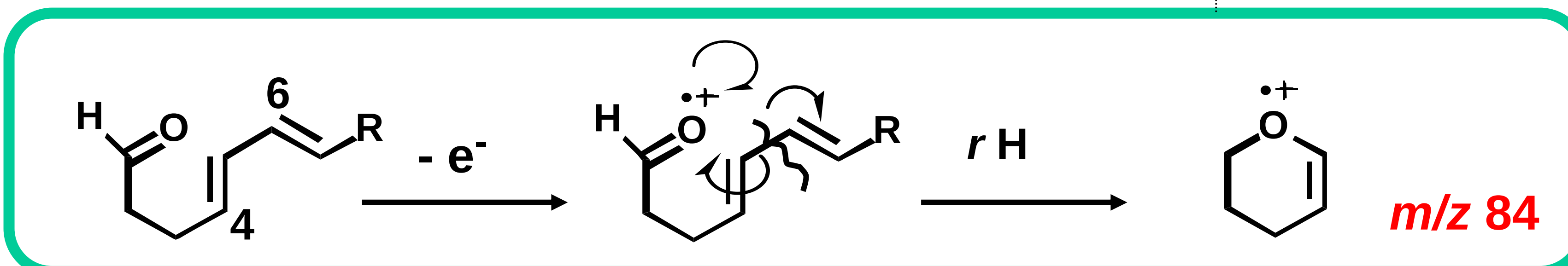
2,4-diene, 3,5-dinen, 4,6-diene
5,7-diene, 6,8-diene, 7,9-diene
8,10-diene

II. GC-MS Analysis ②

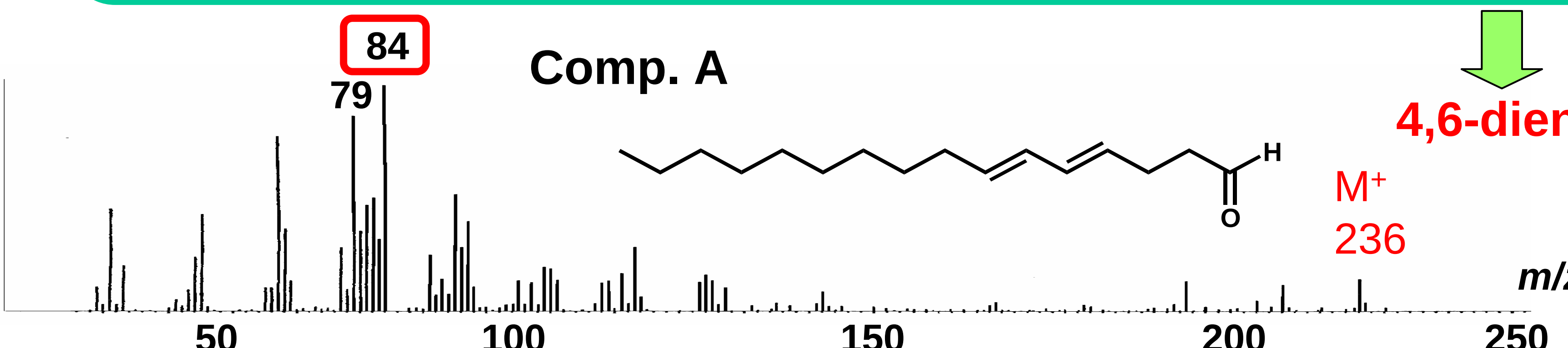
(A) Type I pheromone of the persimmon fruit moth [2]

Base peaks of C-16 dienyl compounds with a conjugated system

	3,5-	4,6-	5,7-	6,8-	7,9-	8,10-	Natural	
Ald	-	84	80	67	67	67	84	← Comp. A
OAc	79	79	79	67	67	67	79	← Comp. B
OH	67	79	79	67	67	67	79	← Comp. C



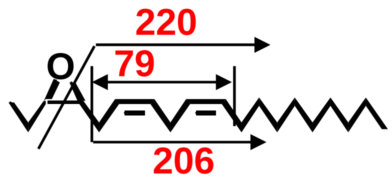
4,6-diene



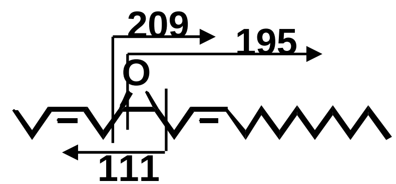
II. GC-MS Analysis ③

(B) Type II pheromone of the Japanese giant looper

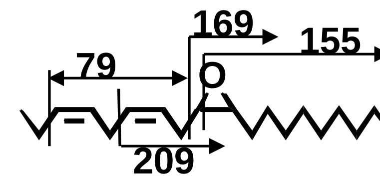
Ascotis selenaria cretacea (Geometridae)



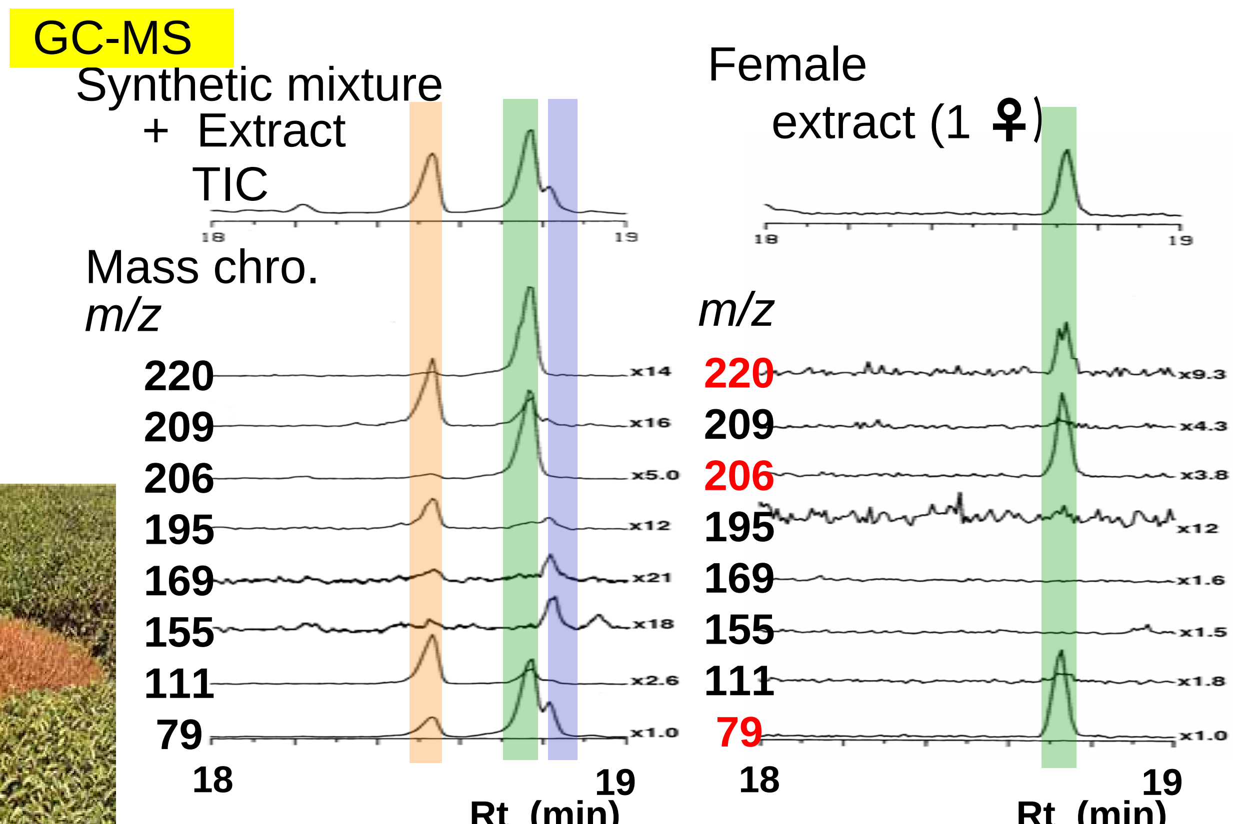
epo3,Z6,Z9-19:H



Z3,epo6,Z9-19:H



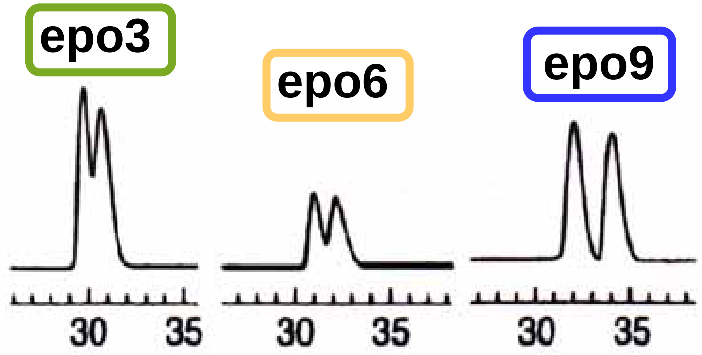
Z3,Z6,epo9-19:H



III. Chiral HPLC Analysis ①

(A) Resolution and stereochemistry of enantiomers

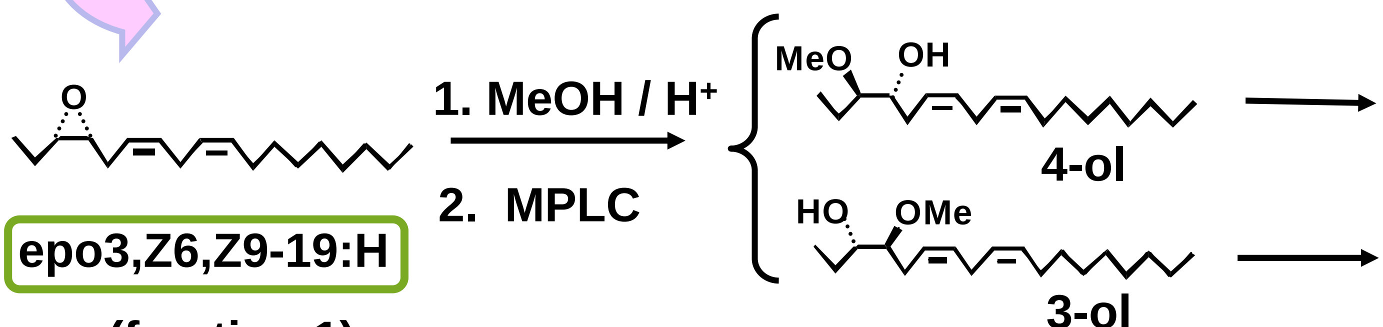
Chiral HPLC



Chiralpak AD

Normal phase

n-hexane +
2-propanol (0.1 %)



epo3,Z6,Z9-19:H

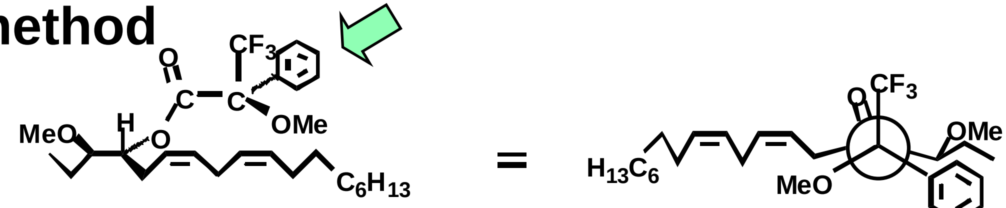
(fraction 1)

esters of (*S*)- and (*R*)-MTPA ester

esters of (*S*)- and (*R*)-MTPA ester

3*S*,4*R*-isomer

Mosher's method

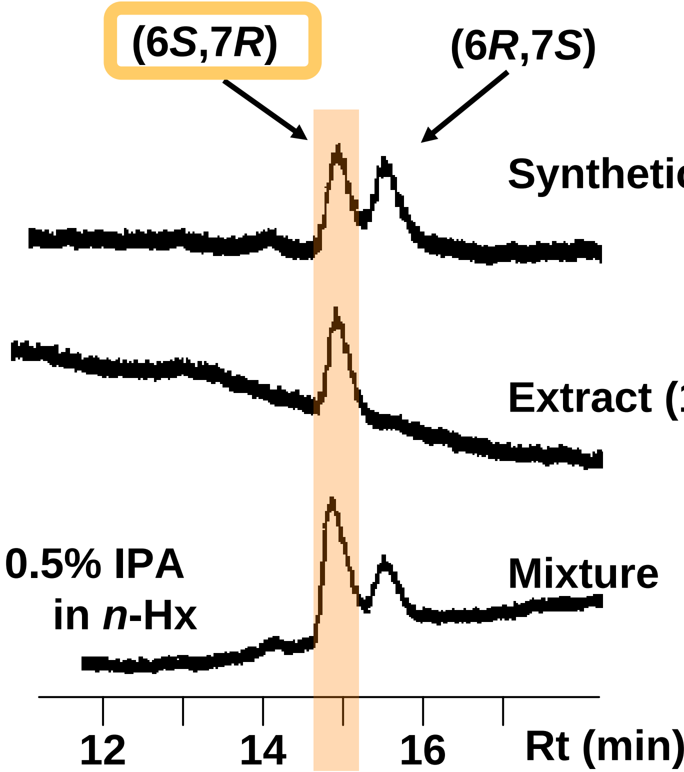


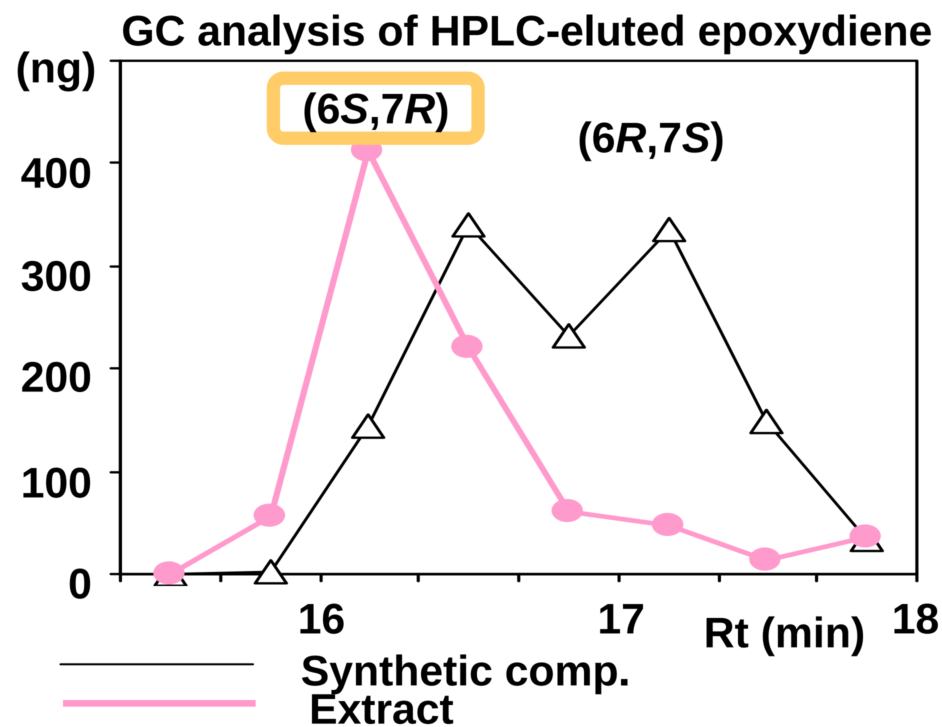
(*S*)-MTPA ester of 4-ol

III. Chiral HPLC Analysis ②

(B) Stereochemistry of the *P. nuda* pheromone (Z3,epo6,Z9-21:H)

Perina nuda (Lymantriidae)
clear-winged tussock moth





Ciral HPLC

Chiralpak AD column

RI detector

IV. Summerry

Identification of Lepidopteran Sex Pheromones

Biological activity	GC-EAD	Routine works ?
Chemical structure	GC-MS	

Yes: ca. 90 % of pheromone ⇨ Type I and Type II comp.

No: Pheromone titer of micro-Lepidoptera ⇨ < 1 pg
Occurrence of novel components

Interesting subjects for the determination

Type I components: double bond positions

New derivatization methods for GC-MS analysis

Type II components: stereochemistry of epoxy ring

Chiral chromatography

Pheromone Studies by Chemical Ecology Laboratory in TUAT

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(4) Biosynthesis and olfactory perception

Experimental confirmation of biosynthetic pathway, substrate specificity of enzymes, and endocrine control system

I. Overview of Applications ①

(A) Application of sex pheromones in IPM programs

① Monitoring tool

To decide the timing of pesticide spraying.

② Mass trapping

Difficult, because of multi-mating of males.

③ Disruption

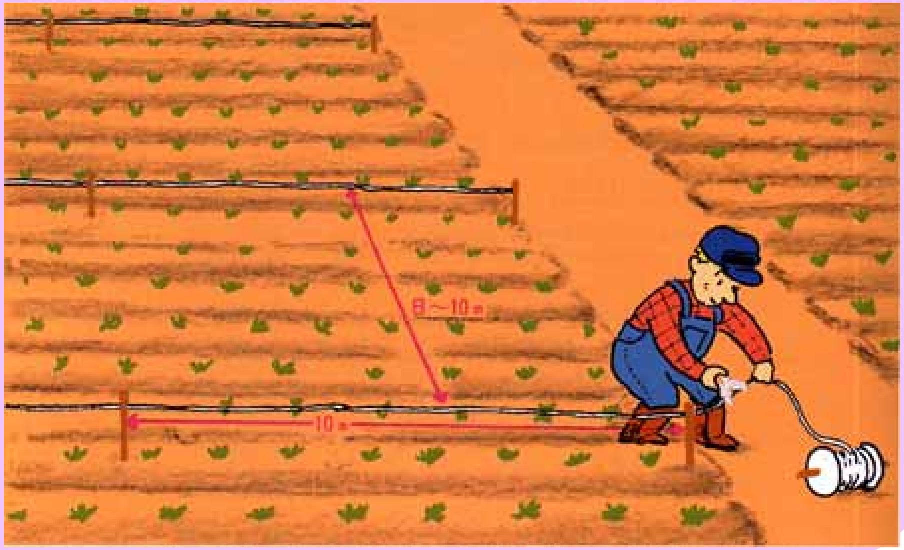
Inhibiting the chemical communication between females and males.

Permeating a field with pheromones

Possible ?

Yes:

Several sex pheromones have registered as an insecticide.



I. Overview of Applications ②

(B) Mating disruption used in the world

Crop	Insect	Country	Applied field (ha) 1997	Applied field (ha) 2002
Cotton	pink bollworm moth	USA	30,000	40,000
		Egypt	328,000	-
		Israel	8,000	5,000
Apple, Pear	coddling moth	USA	13,200	63,000
Grape	grapevine moth grape moth	EU	25,000	73,000
Tea	small tea tortrix	Japan	400	500
Vegetable	diamondback moth	Japan	1,000	2,000
Forest	gypsy moth	UAS	10,000	150,000

K. Ogawa、*Bio-control*、**2**：18 (1998)

I. Overview of Applications ③

(B) Mechanism of Mating Disruption: Unclear

① **Wasteful exiting and attraction**
② **Mask of virgin females**
  by a higher level of the synthetic pheromone
  by modification of the natural mixing ratio

A copy of the natural pheromone blend

→ **Best attractant**
→ **Good disruptant ?**

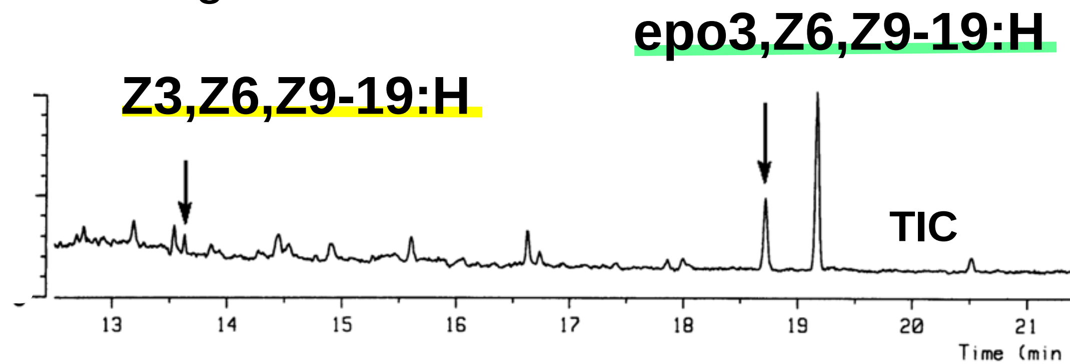
"Hamaki-con" Z11-tetradecenyl acetate

Tea	***Adoxophyes honmai*** **sp. nov.**
	Homona magnanima
Apple	***Adoxophyes orana***
	Archips breviplicanus
	Archips fuscocupreanus

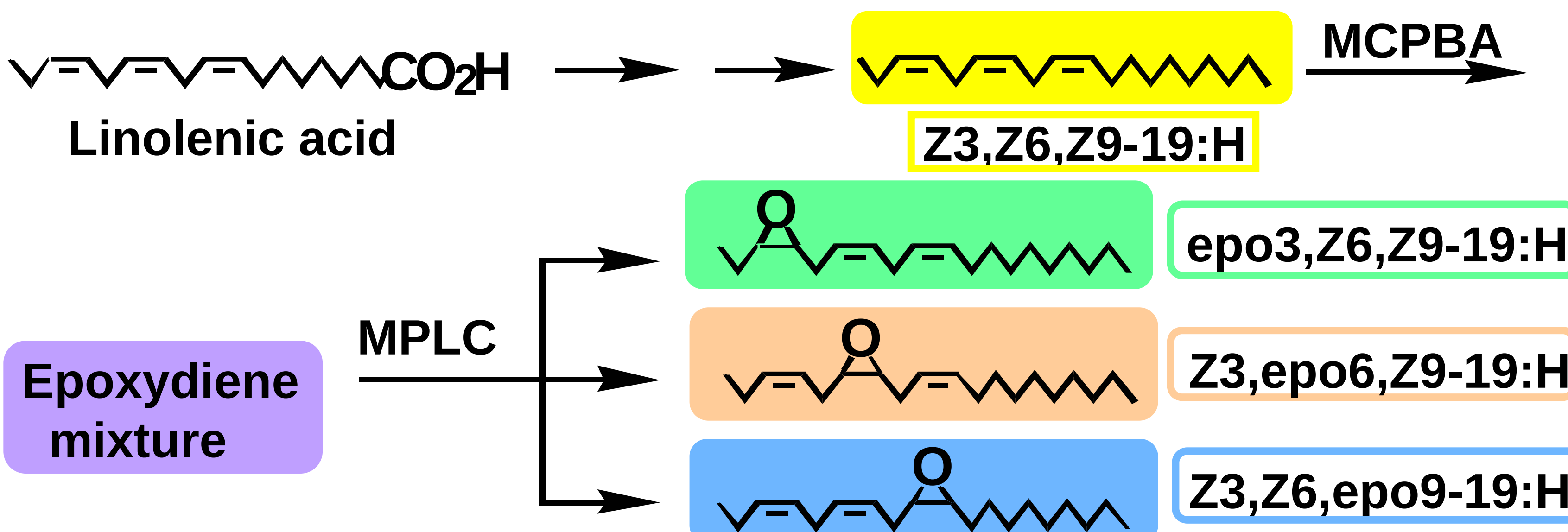
II. Disruption with epoxyalkenyl pheromones ①

Japanese giant looper

GC-MS analysis of the pheromone gland extract



(A) Synthesis of pheromone components and analogs



II. Disruption with epoxyalkenyl pheromones ②

(B) Formulation of synthetic compounds

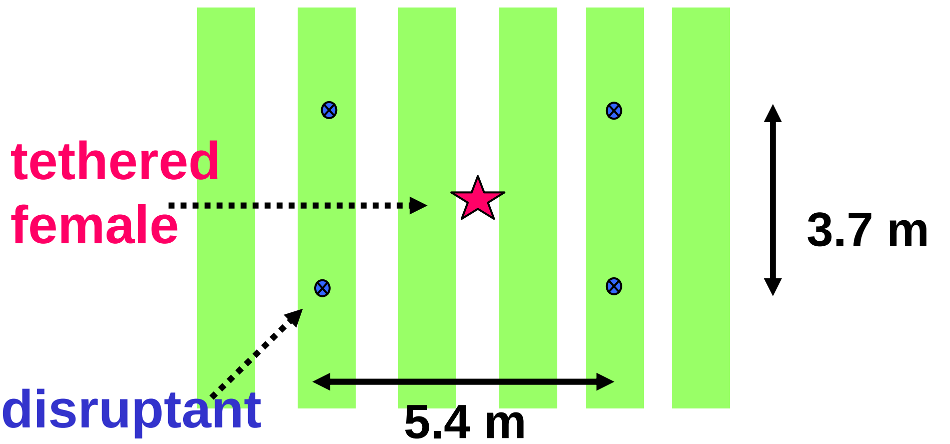
Polyethylene dispensers were prepared by Shin-Etsu Chemical Co., Ltd.

Dispenser was fixed to a tea branch after labeled with a paper tag.

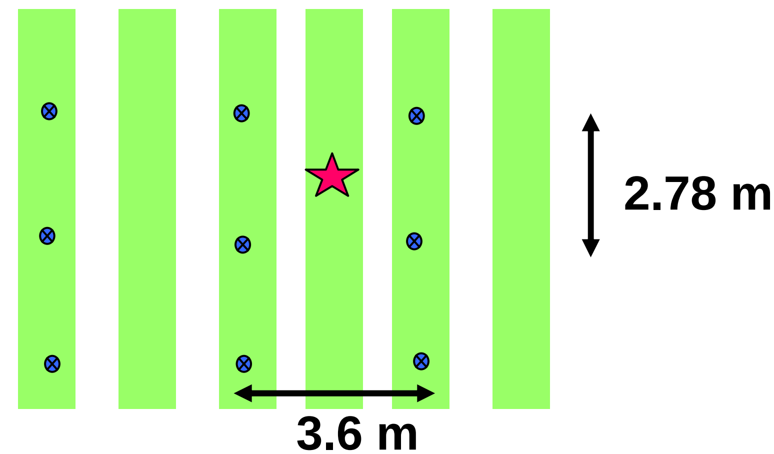
II. Disruption with epoxyalkenyl pheromones ③

(C) Field evaluation of disruptant

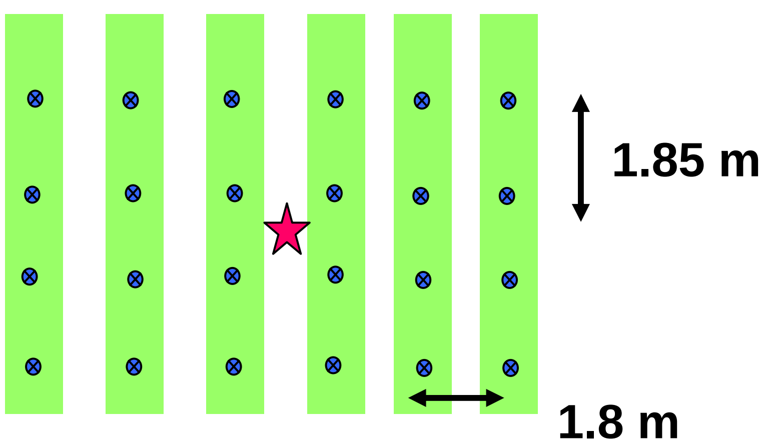
(a) 500 tubes / ha (16 tubes / 180 m^2)



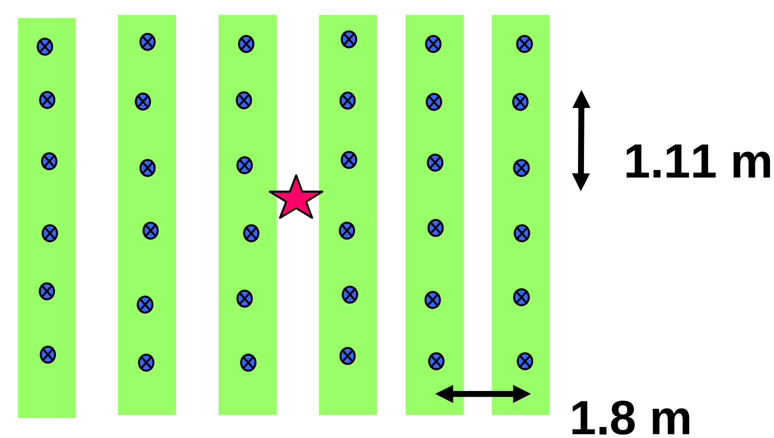
(b) 1000 tubes / ha (16 tubes / 90 m^2)



(c) 3000 tubes / ha (48 tubes / 82 m^2)



(d) 5000 tubes / ha (48 tubes / 70 m^2)



Evaporation; triene (0.546 mg/tube/day), epoxydiene (0.386 mg/tube/day)

II. Disruption with epoxyalkenyl pheromones ④

(D) Mating inhibition in a field treated with disruptants

Mating Ratio of *A. s. cretacea* Females Tethered in a Tea Garden, which was Permeated with Triene or an Epoxydienes Mixture Released from Dispensers

Dispenser (N / ha)	(A) Triene: No. of females Tethered	(A) Triene: No. of females Mated	(A) Triene: Mating ratio (%)	(B) Epoxydiene mixture: No. of females Tethered	(B) Epoxydiene mixture: No. of females Mated	(B) Epoxydiene mixture: Mating ratio (%)
0	11	11	100	14	14	100
500	10	6	60	14	4	29
1000	9	6	67	14	1	7
3000	10	8	80	12	0	0
5000	10	4	40	12	0	0

Tested from Sept. 7 to 18, 1999.

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I. Proposed pathways for lepidopteran sex pheromones

Type I (Acetate of unsaturated fatty alcohol)

Anadevidia peponis

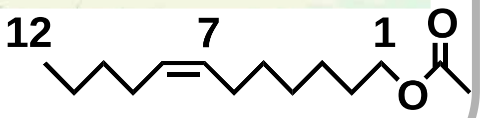
Acetyl CoA

16:Acyl → Z11-16:Acyl (Δ11-desaturation)

Z11-16:Acyl → Z9-14:Acyl → Z7-12:Acyl (β-oxidation)

Z7-12:Acyl → Z7-12:OAc (reduction, acetylation)

pheromone gland



Type II (Epoxide of unsaturated hydrocarbon)

A. s. cretacea

Z9,Z12,Z15-18:Acyl

↓

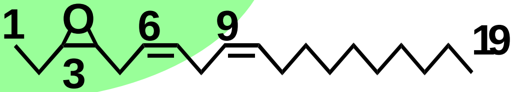
Z11,Z14,Z17-20:Acyl → Z3,Z6,Z9-19:H (decarboxylation)

↓

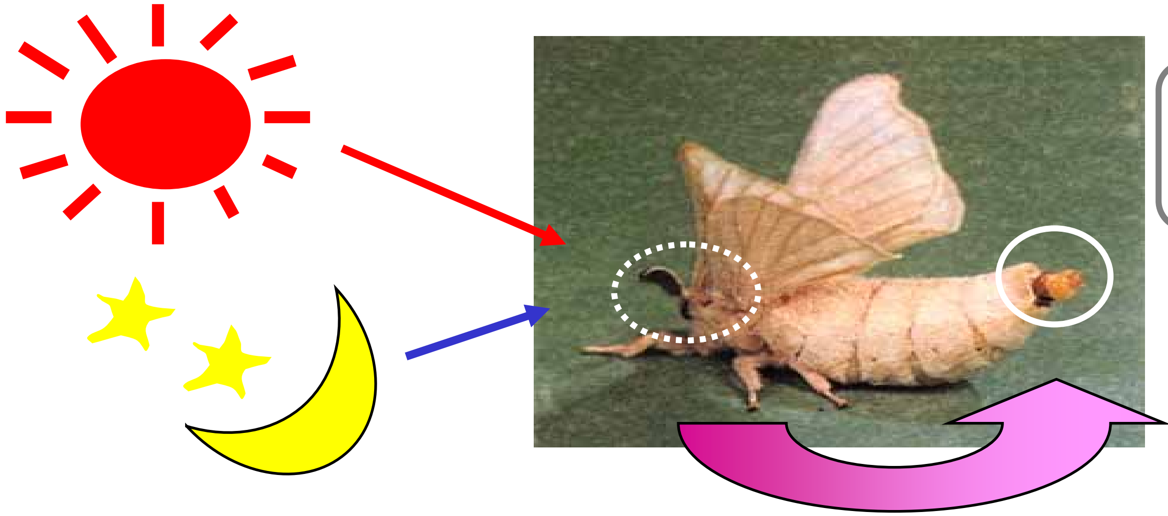
oenocyte

Z3,Z6,Z9-19:H → epo3,Z6,Z9-19:H (epoxidation)

pheromone gland

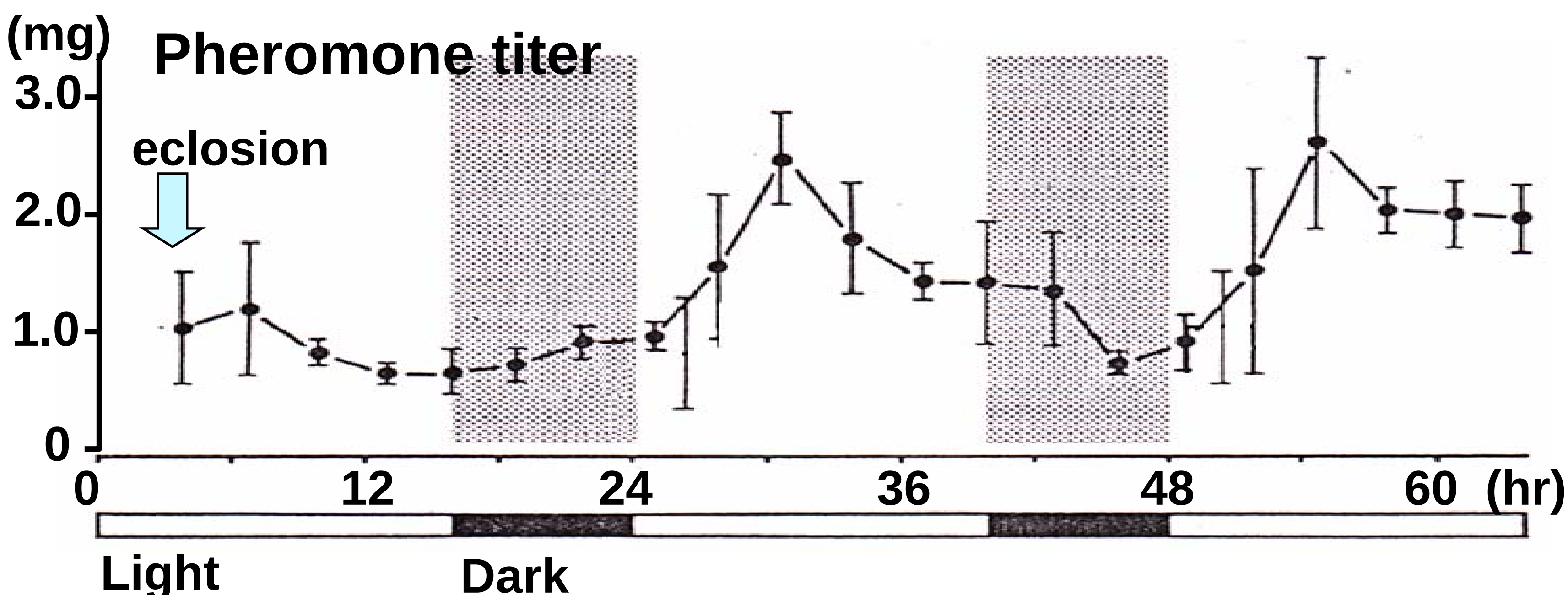


II. Endocrine control of pheromone production

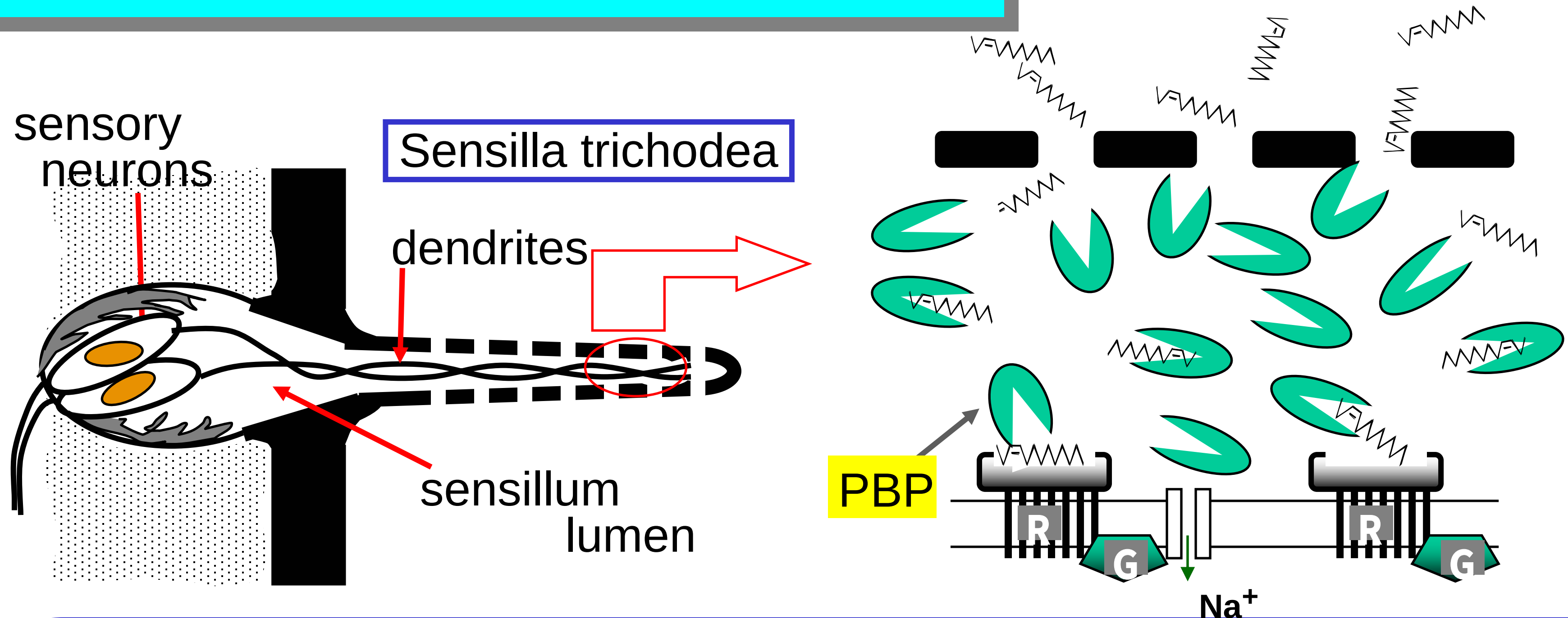


pheromone biosynthesis

PBAN
pheromone biosynthesis activating neuropeptide



III. Mechanism of perception



Vogt & Riddiford, 1981. *Nature*, **293**: 161

Photo-labeling experiment ➡ 15 KDa protein (10 mM)

Pheromone-Binding Protein (PBP)

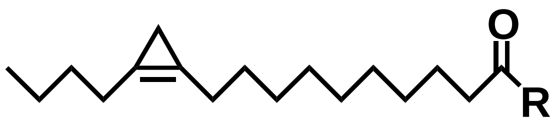
Receptor ➡ unknown

Raming *et al*., 1989. *FEBS Lett.*, **256**: 215

Cloning of cDNA, Size: 15 KDa, Occurrence: in sensilla trichodea of male antennae, Function: transporter of a lipophilic pheromone to the receptor

IV. Inhibitors of pheromone biosynthesis

a) Inhibitors of desaturase

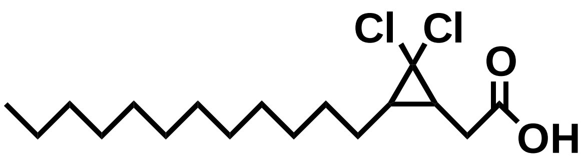


R = OH, NH_2

Arsequell *et al.*, 1989. *Insect Biochem.*, **19**: 949.

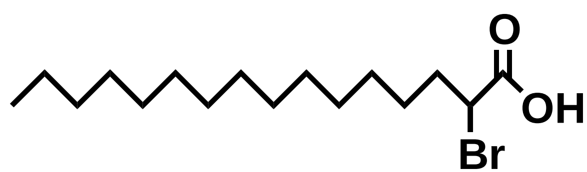
Ando *et al.*, 1995. *J. Pestic Sci.*, **20**: 25.

b) Inhibitors of β-oxidase



Hernanz *et al.*, 1997. *J. Lipid Res.*, **38**: 1988.

Rosell *et al.*, 1992. *Insect Biochem. Mol. Biol.*, **22**: 679.



Are these studies applicable to pest managements ?

Fundamental sciene ⇔ Application

Evolution ⇐ Diversity of insect species

Synchronize { **Sex pheromone production in ♀** / **Reception by ♂** }

Understanding at the level of proteins and genes

Acknowledgments

Co-workers

Drs. K. TSUCHIDA and H NAKA

Pheromone identification from the persimmon fruit moth

Drs. S. WAKAMURA and N. ARAKAKI

Pheromone identification from the clear-winged tussock moth

Drs. F. MOCHIDUKI, T. FUKUMOTO and K. OHTANI

Mating disruption of the Japanese giant looper

Chemical Ecology Laboratory of TUAT

Dr. X.-R. QIN
Dr. WITJAKSONO
Dr. S. INOMATA
Dr. M. YAMAMOTO
Dr. A. ONO
Dr. H. YAMAZAWA

T. MIYAMOTO
S. SHIMADA
Y. TAKEUCHI
N. NAKAJIMA
W. WEI
T. NISHIDA
L. V. VANG
T. MURAKAWA