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GC-FT-IR Analyses of Sex Pheromones Secreted by Nettle Moths

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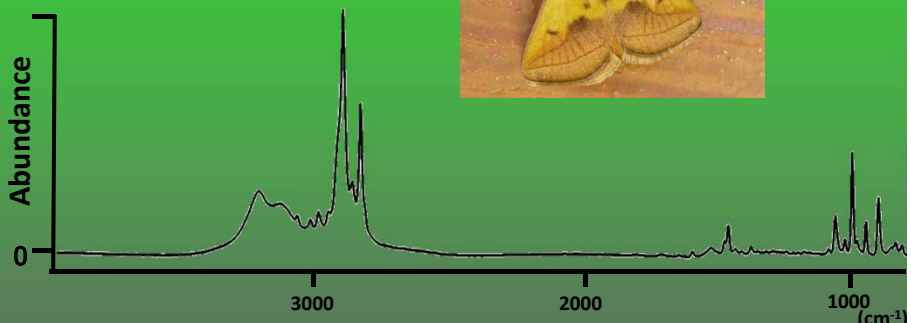


Lepidopteran sex pheromones

Identified from females of more than 630 species

General procedures of the identification

1. GC-EAD to find an active component
2. GC-MS to determine the chemical structure

Functional group → OH

Carbon chain length → 16

Double bond

Number → 2

Position → 10, 12

Configuration → E, Z

Functional group → OH

Carbon chain length → 16

Double bond

Number → 2

Position → 10, 12

Configuration → E, Z

Determined by the IR spectrum,
if a large amount of the pure pheromone is available.

IR analysis is not utilized, because
the species-specific pheromones are composed of multi components,
which are produced around ng level.

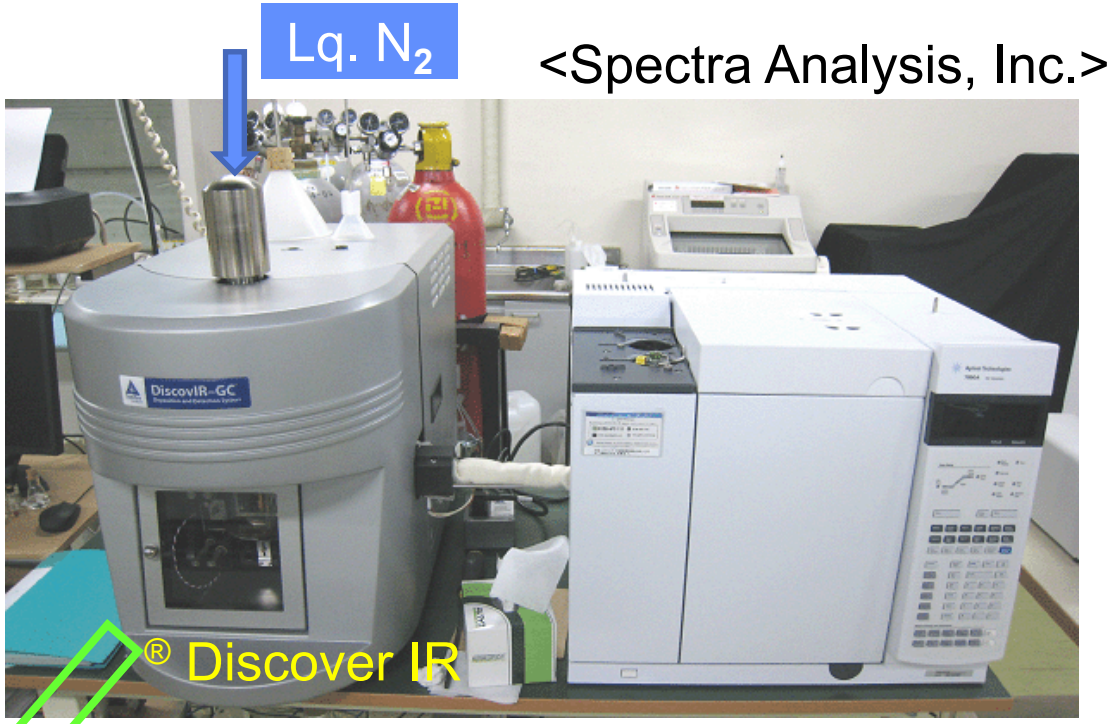
GC-FT-IR

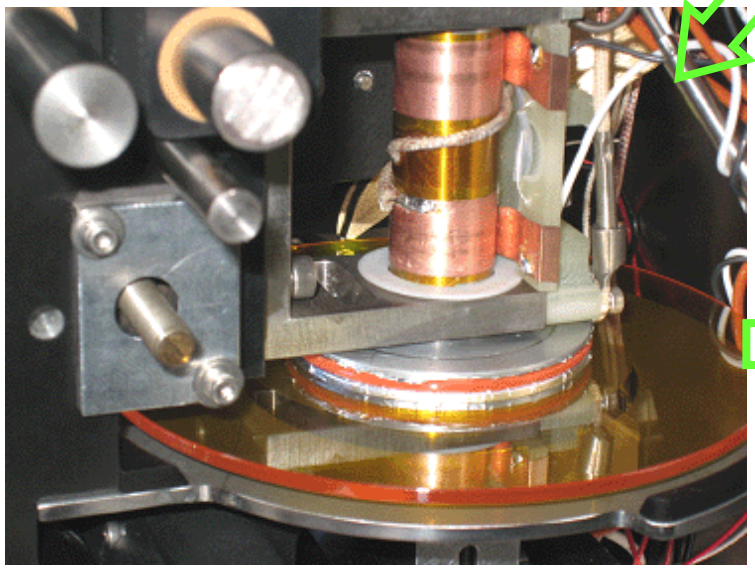
Capillary GC
- ⇨ High separation

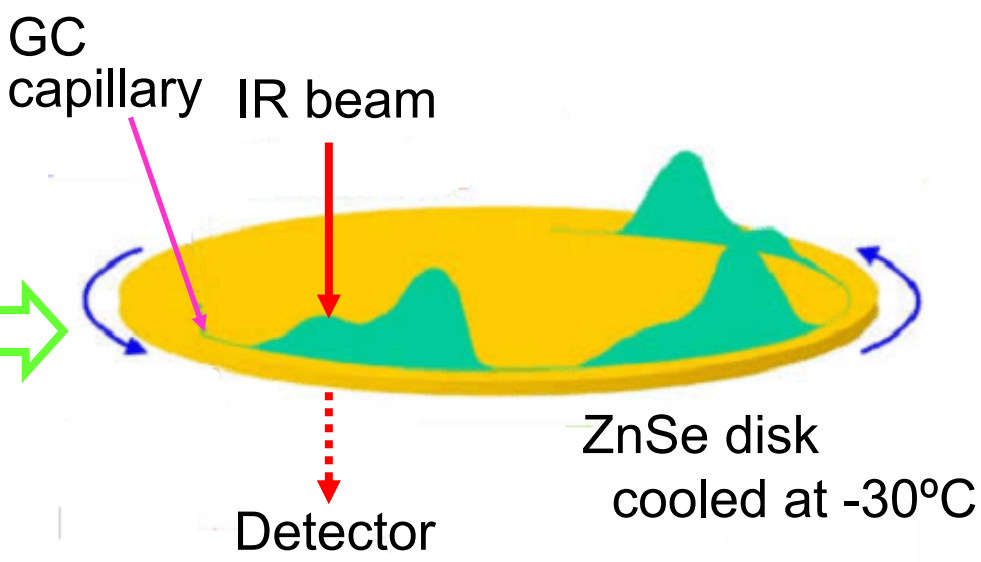
FT-IR (solid phase)
- ⇨ High sensitivity

Familiarized spectrum

<Spectra Analysis, Inc.>







Identification of terminal conjugated dienes

[Limacodidae] Nettle moths

in Japan

Parasa lepida

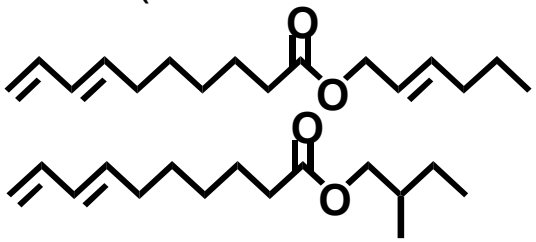
(Azharul *et al.*, 2009. *B. B. B.*, **73**: 1156-1162)

Oil palm defoliators

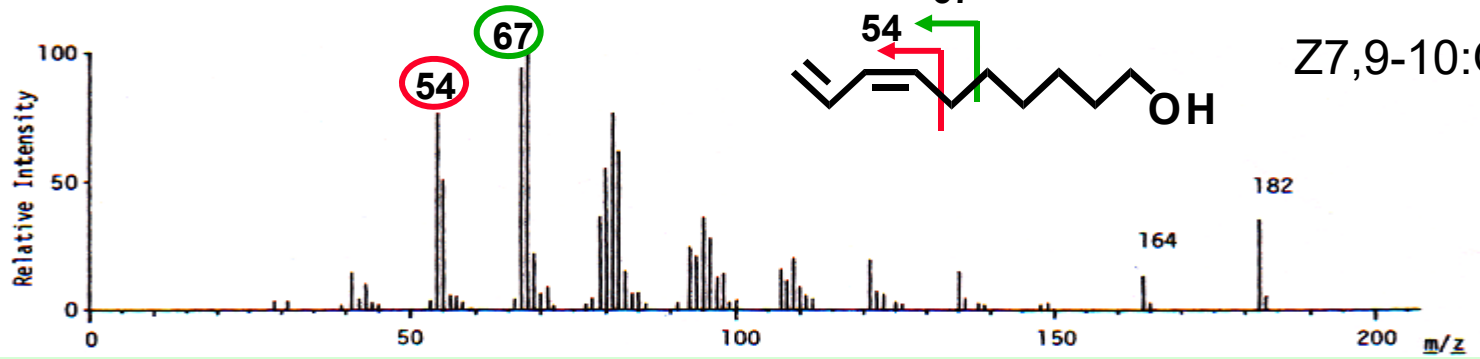
Setothosea asigna (Sasaerila *et al.*, 1997) in Indonesia

E9,11-12:Ald

Darna trima (Sasaerila *et al.*, 2000) in Malaysia



E7,9-10:Acyl derivatives



Terminal conjugated structure is easily determined by fragment ions at *m/z* 54 and 67.

How to know the configuration ? By GC ?

Separation of terminal conjugated dienes

(1) GC

Column: DB-23
(0.25 mm X 30 m)

Z7,9-10:OH

E7,9-10:OH

Synthetic mixture

Z:E = 10:4

Z:E = 10:1

Natural pheromone

10.0

15.0

t_R (min)

(2) HPLC

Column: ODS
Eluent: 45 % H_2O in MeOH

Z7,9-10:OH

E7,9-10:OH

Z:E = 10:4

Z:E = 10:1

20.0

25

t_R (min)

The females exclusively produce the (*Z*)-isomer.
Reversed-phase HPLC is better than GC.
(Azharul *et al*., 2009)

Other detector of capillary GC? → FT-IR

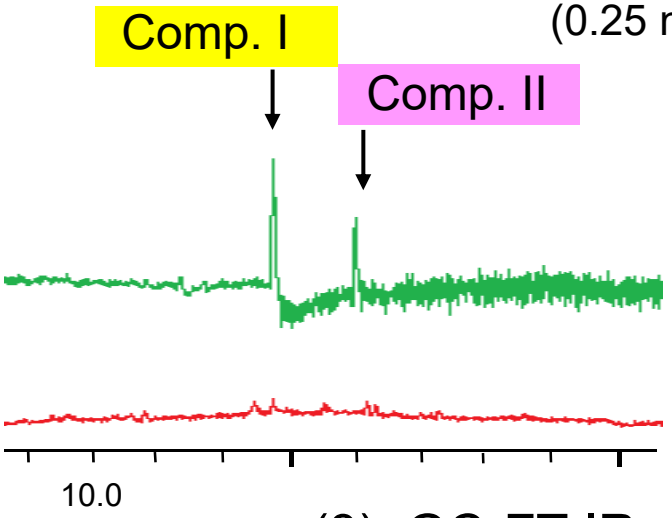
Sex pheromone of the oriental moth

Monema flavescens
(Limacodidae)

Wintering
in cocoon

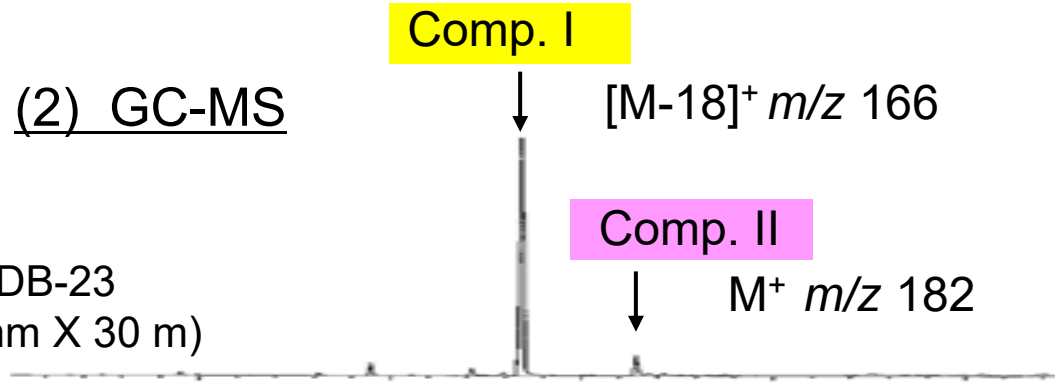
Eclosion in July
(Univoltine)

(1) GC-EAD



(2) GC-MS

Column: DB-23
(0.25 mm X 30 m)



Comp. I

DMDS derivative → 8-monoene

Comp. II

Mass spectrum → 7,9-diene

(3) GC-FT-IR

Configuration of the double bonds ??

GC-FT-IR analysis

Comp. I

Comp. II

Crude pheromone extract (20FE)

Column: HP-5
(0.25 mm X 30 m)

Peak chromatogram

Disk temperature: -30ºC
Disk speed: 3 mm/min

Abundance

0

5.0 10.0 15.0 (min) 20.0

C-H bending vibrations

Di-substituted monoene

trans 970-960 cm^{-1}

cis 730-675 cm^{-1}

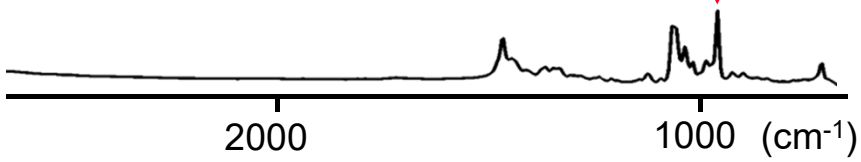
gem 895-885 cm^{-1}

Conjugated diene

Similar absorption

E8-10:OH

966 cm^{-1}

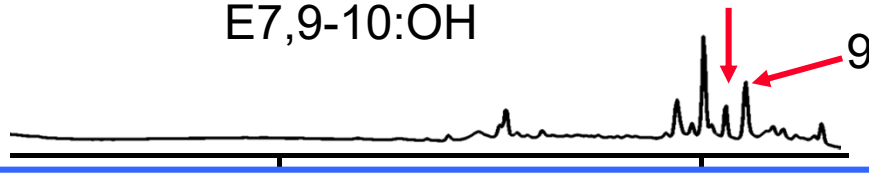


(*E*) -isomer

E7,9-10:OH

951 cm^{-1}

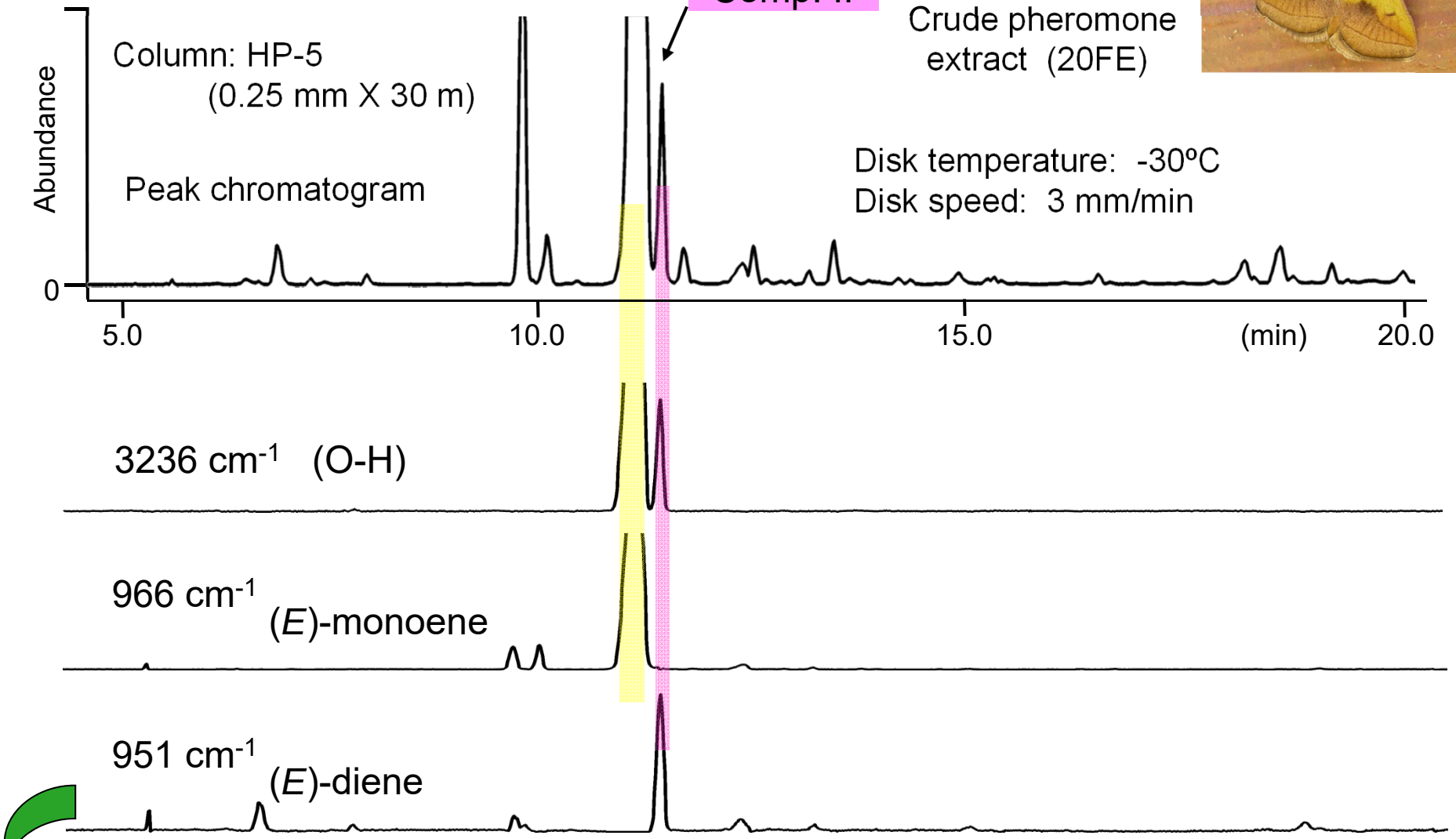
903cm^{-1}



Discover IR-GC®

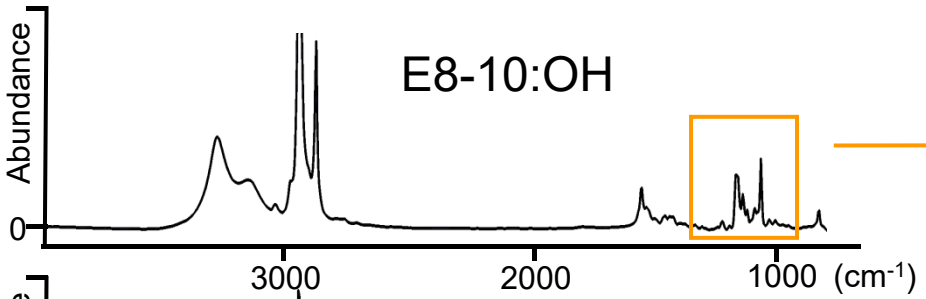
Recording range: 4000-700 cm^{-1} → (*Z*)-Isomers are not directly identified.

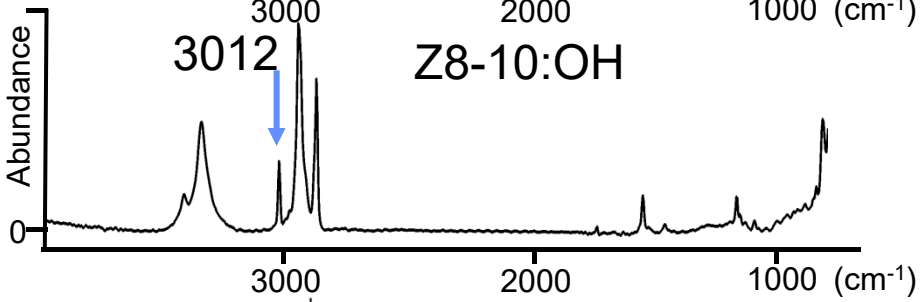
Band chromatograms

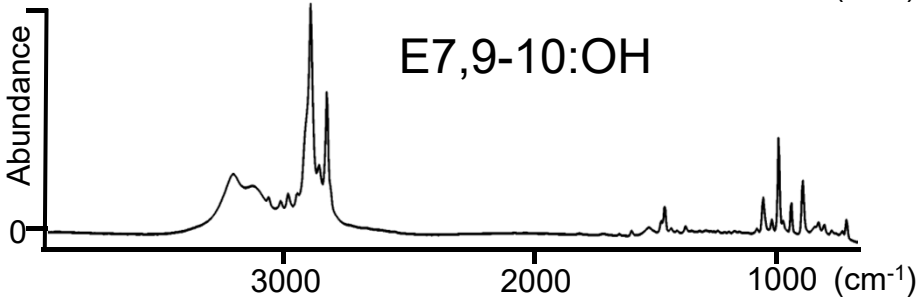


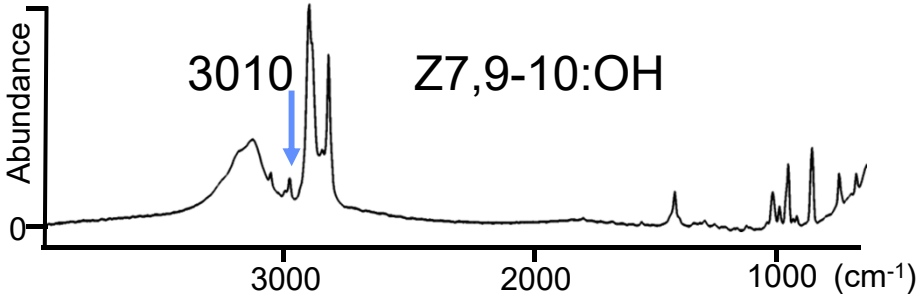
These band chromatograms made clear the two different components with the same configuration.

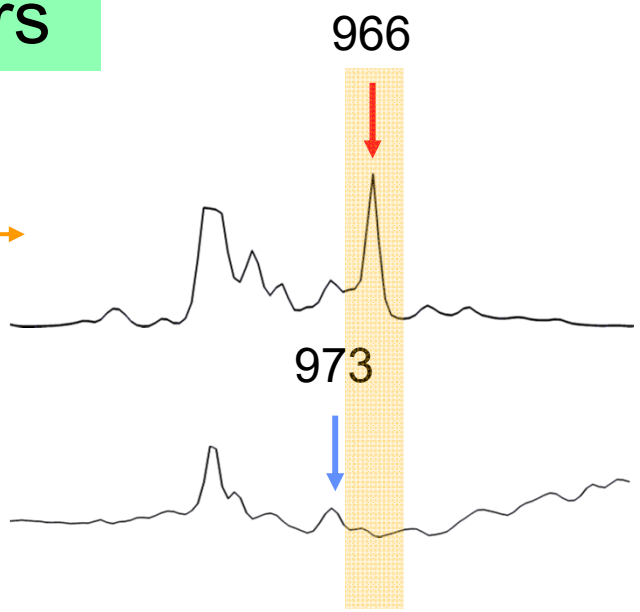
IR spectra of (*E*)- and (*Z*)-isomers

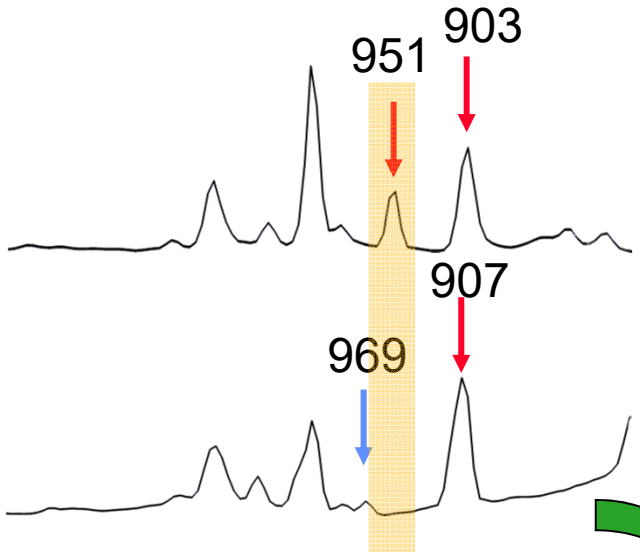












Configurations are confirmed by band chromatograms at 966 and 951 cm^{-1}.

GC-FT-IR analysis of natural pheromones

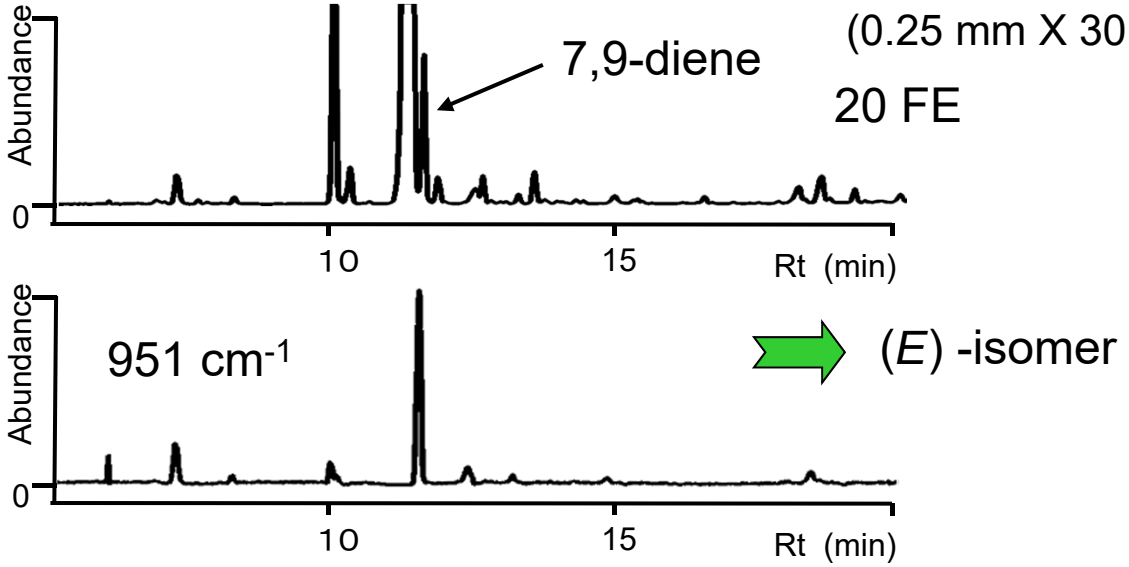
Oriental moth

Monema flavescens

Peak chromatogram

Band chromatogram

Column: HP-5
(0.25 mm X 30 m)

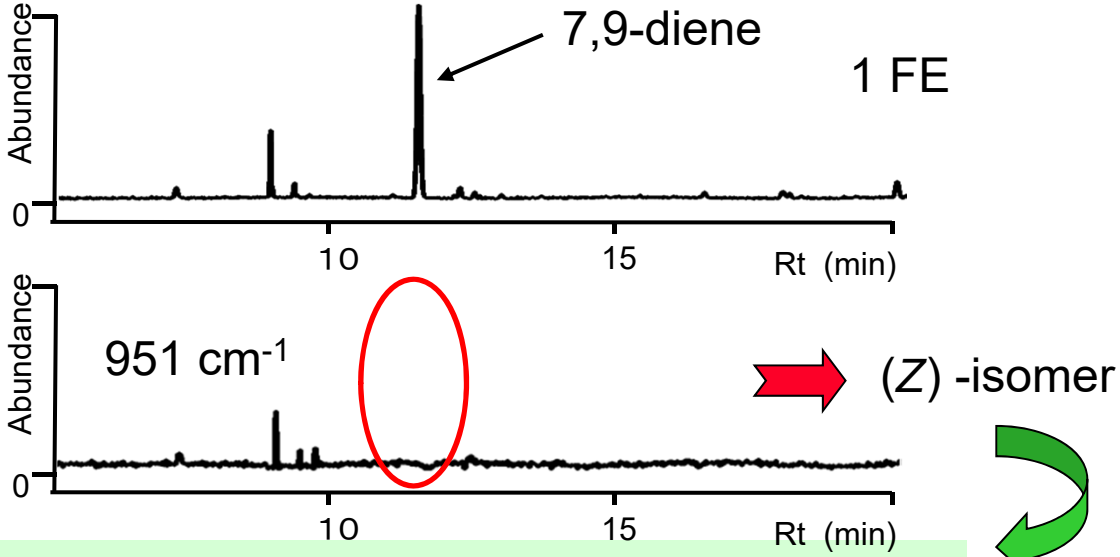


Green nettle moth

Parasa lepida

Peak chromatogram

Band chromatogram



(*Z*)-Configuration is confirmed by absence of absorption at 951 cm^{-1}.

Field evaluation of synthetic pheromones

Oriental moth

Monema flavescens

June 29 to July 16, 2012

Lure contents (mg/septum)		Captured males
E8-10:OH	E7,9-10:OH	/ trap
1.0	0.0	0
0.9	0.1	4.5 ± 0.6 a
0.5	0.5	0.8 ± 0.3 b
0.1	0.9	0
0.0	1.0	0
0.0	0.0	0

New components of Type I pheromones

Green nettle moth

Parasa lepida

Aug. 21 to Sept. 3, 2007

Lure contents (mg/septum)		Captured males
Z7,9-10:OH	E7,9-10:OH	/ trap
0.50	0.00	9.5 ± 0.3 a
0.45	0.05	4.5 ± 0.8 b
0.25	0.25	1.0 ± 0.3 c
0.00	0.50	1.0 ± 0.3 c
0.00	0.00	0

Further studies

Identification of sex pheromones secreted by Limacodidae species

About 30 species of the nettle moths inhabit Japan

Conservation of the terminal conjugated dienyl structure ??

Structural diversity of the female pheromones ??

Mechanism of reproductive isolation ??

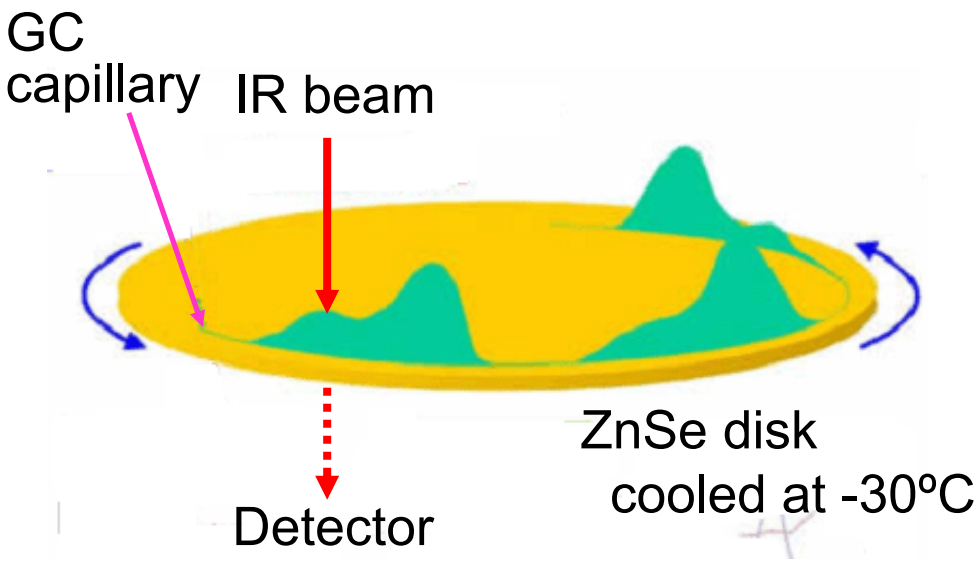
(geographical factor, moving area, northern limit of inhabitancy)

Biosynthesis of the terminal conjugated dienenes

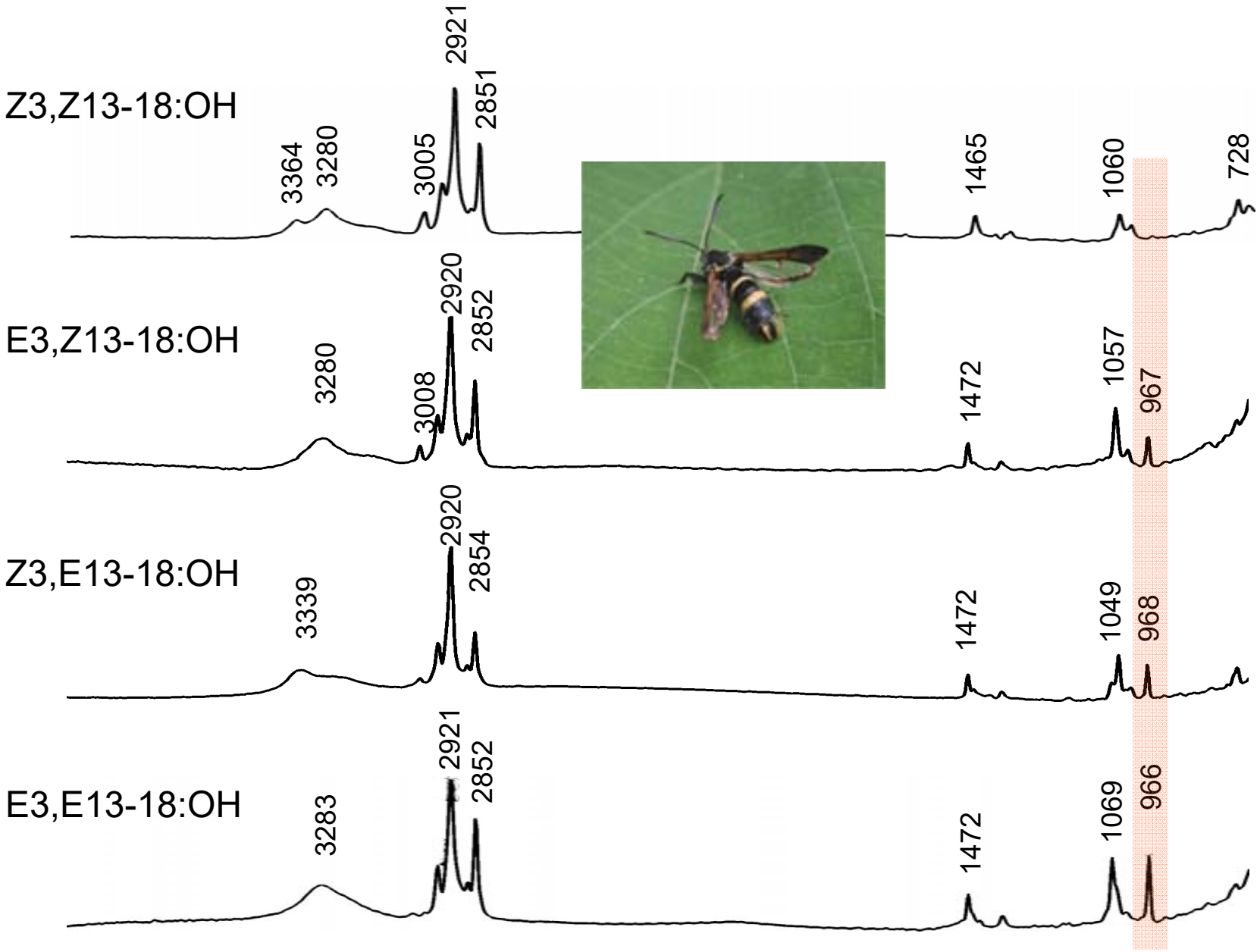
Applications of GC-FT-IR

Isolated dienes

Conjugate dienes



GC-FT-IR analysis of synthetic 3,13-dienes



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Thank you for your attention!!

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