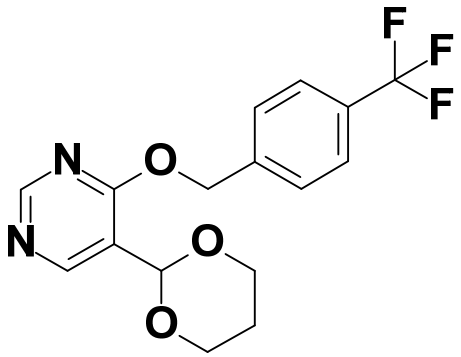
Mode of Action of Benzpyrimoxan

Effects on Chithin Biosynthesis and Ecdysteroids Titer

Nihon Nohyaku Co., Ltd.

Chemical structure and Biological properties

■ Structure / name



- Common name：Benzpyrimoxan
- Benzpyrimoxan belongs to a novel chemical class as “benzyloxypyrimidines”, that is different from existing insecticides.

■ Biological property

- Highly effective against planthoppers in paddy fields.
- Also effective in populations that have developed resistance to existing insecticides.
- Low impact on beneficial insects and non-target arthropods.

■ Schedule (solo formulation product)

- Application for registration：2019/Feb. (Japan, India)
- Registration approval：2020/Sep. (Japan), 2021/Apr. (India)

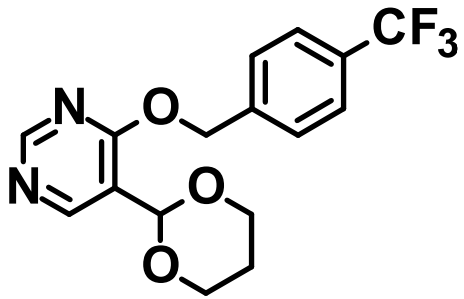
Launched this May as a new hopper-cide in the paddy field (foliar spray use, Japan).

Characteristics of benzpyrimoxan in action and comparison with existing insecticides

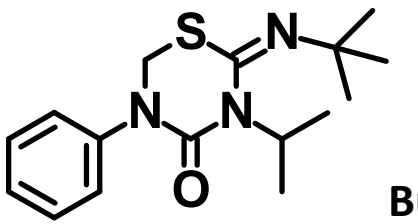
◆ Characteristics in action

- ➢ Inhibition of molting
- ➢ Only acts on nymphs

●We conducted comparison study between benzpyrimoxan and buprofezin, the only existing insecticide for rice that inhibits molting of hoppers

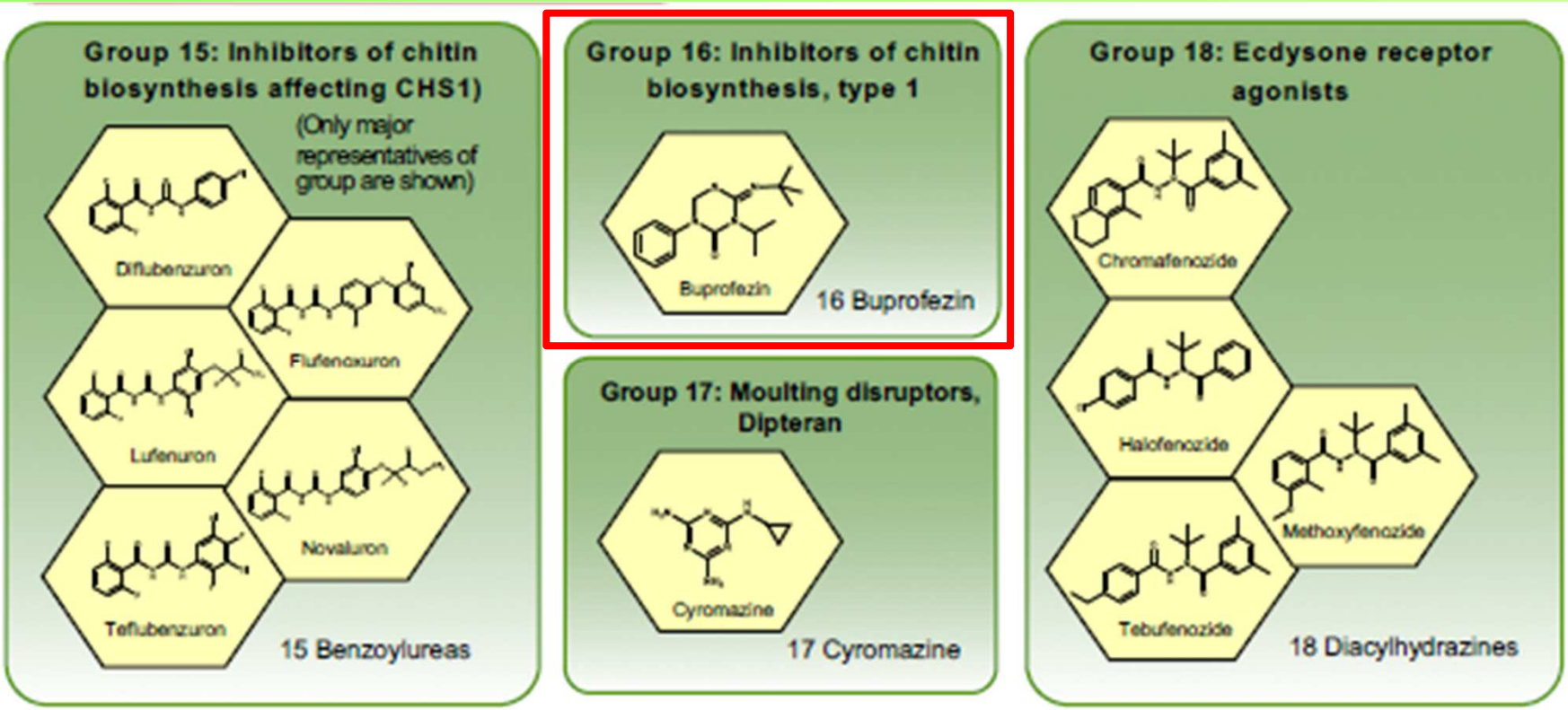


Benzpyrimoxan



Buprofezin

IRAC classification of buprofezin



https://irac-online.org/ poster (v7.4)

IRAC classification of buprofezin ;

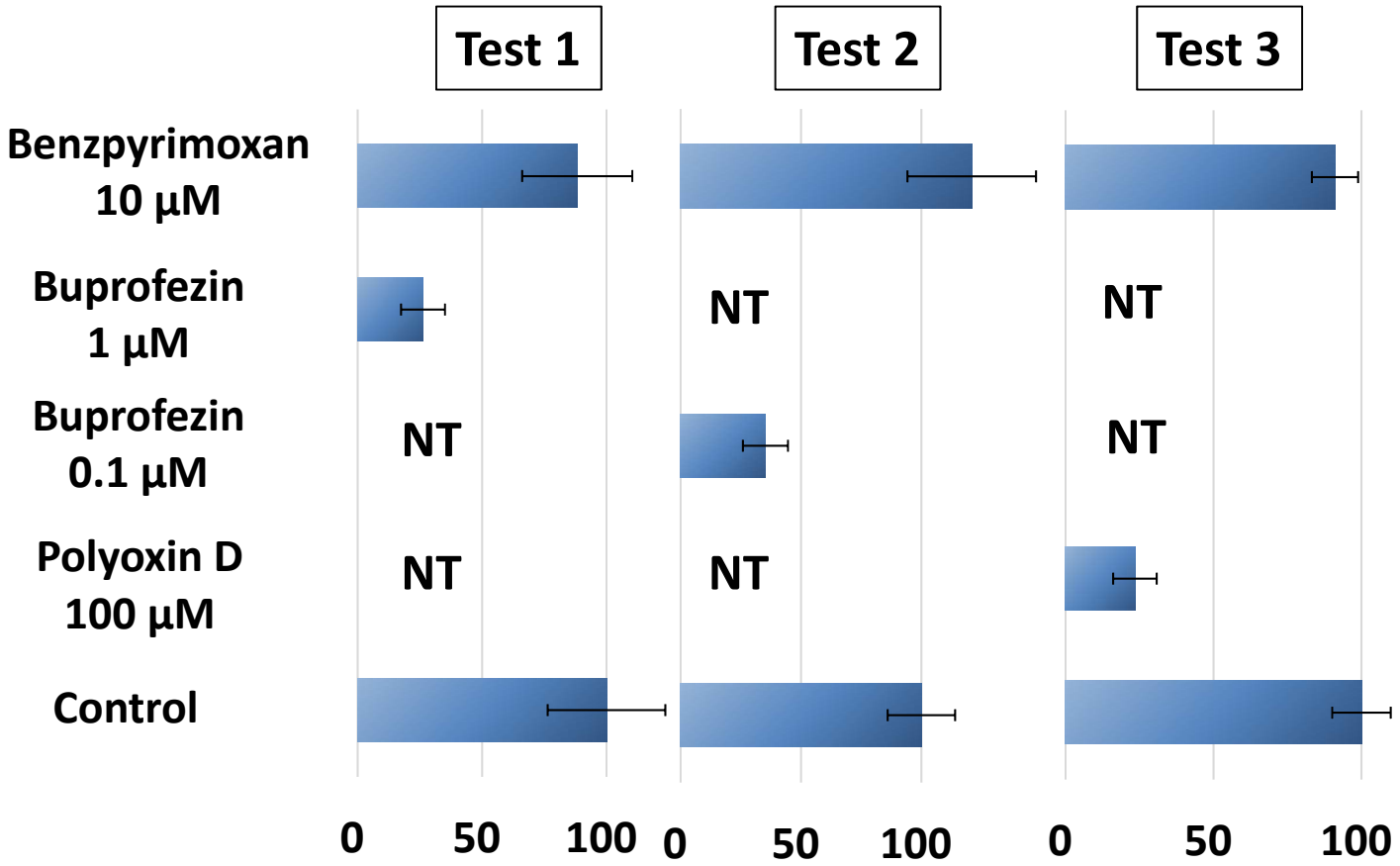
Group 16 Inhibitors of chitin biosynthesis, type1

➡ **The effect of benzpyrimoxan on chitin biosynthesis was studied**

Chitin ; a long-chain polymer of N-acetylglucosamine, a primary component the exoskeletons of insects

Results of chitin biosynthesis assay

Radioactivities of Chitin fractions of brown planthopper (% of Control)



Summary of method

Sampling individuals just after molting

↓

Dissected body and only abdominal integuments were collected

↓

Integuments & substrate ([^{3}H]N-acetylglucosamine) were reacted in the presence of compounds.

↓

Samples were treated with hot KOH, the filtered residue was used as the chitin fraction and the radioactivity was measured.

n = 3, Ave. & SD were represented
NT: Not treated

- Buprofezin and Polyoxin D inhibited chitin biosynthesis, while Benzpyrimoxan did not.

→ **Benzpyrimoxan does not directly inhibit chitin biosynthesis, and the mechanisms of action of benzpyrimoxan and buprofezin are different.**

Estimation of MoA from action characteristics

◆ Action Characteristics

- ➢ Inhibition of molting
- ➢ Only acts on nymphs

→ ● Affecting the regulation of molting or juvenile hormones?

◆ The molting process itself occurs.

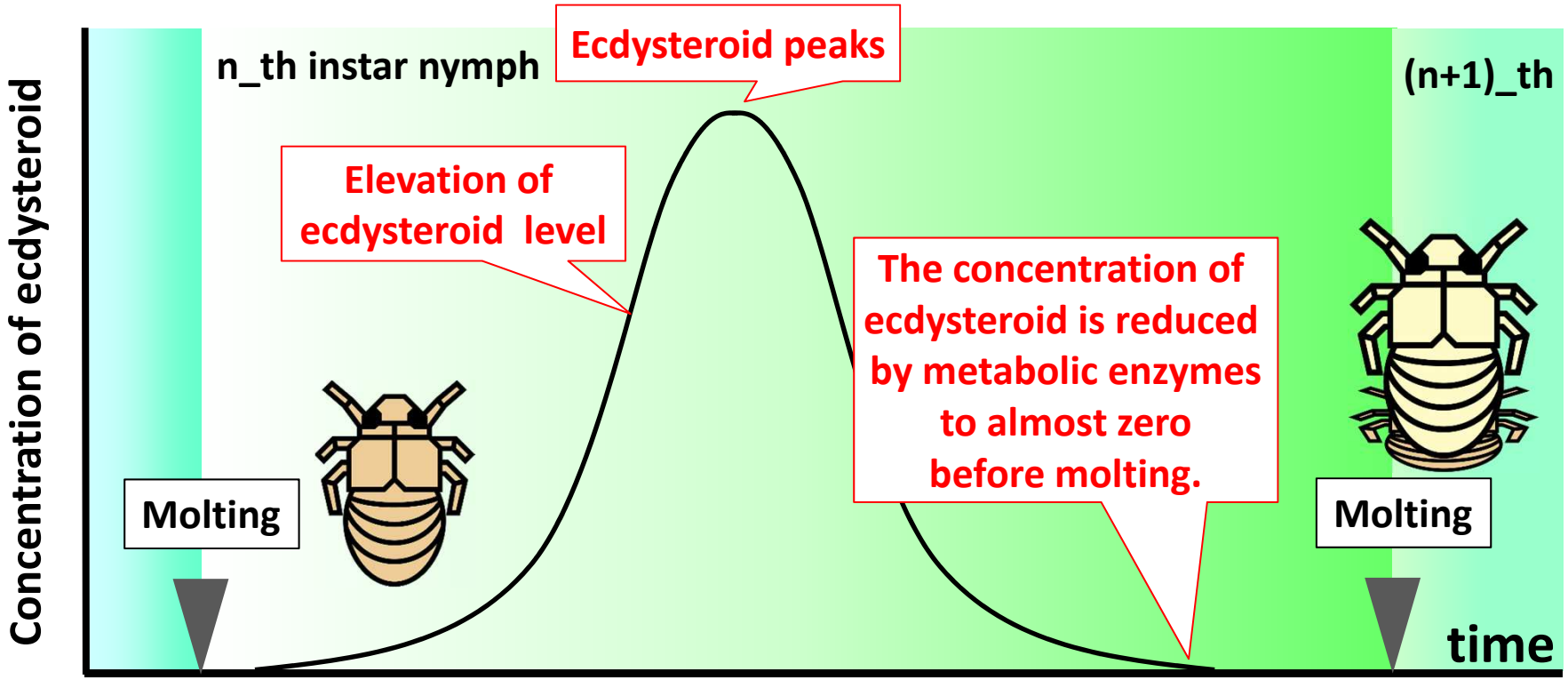
➡ Molting hormone is present in the body. (Biosynthesis of hormone is not completely stopped)

◆ No precocious metamorphosis was observed.

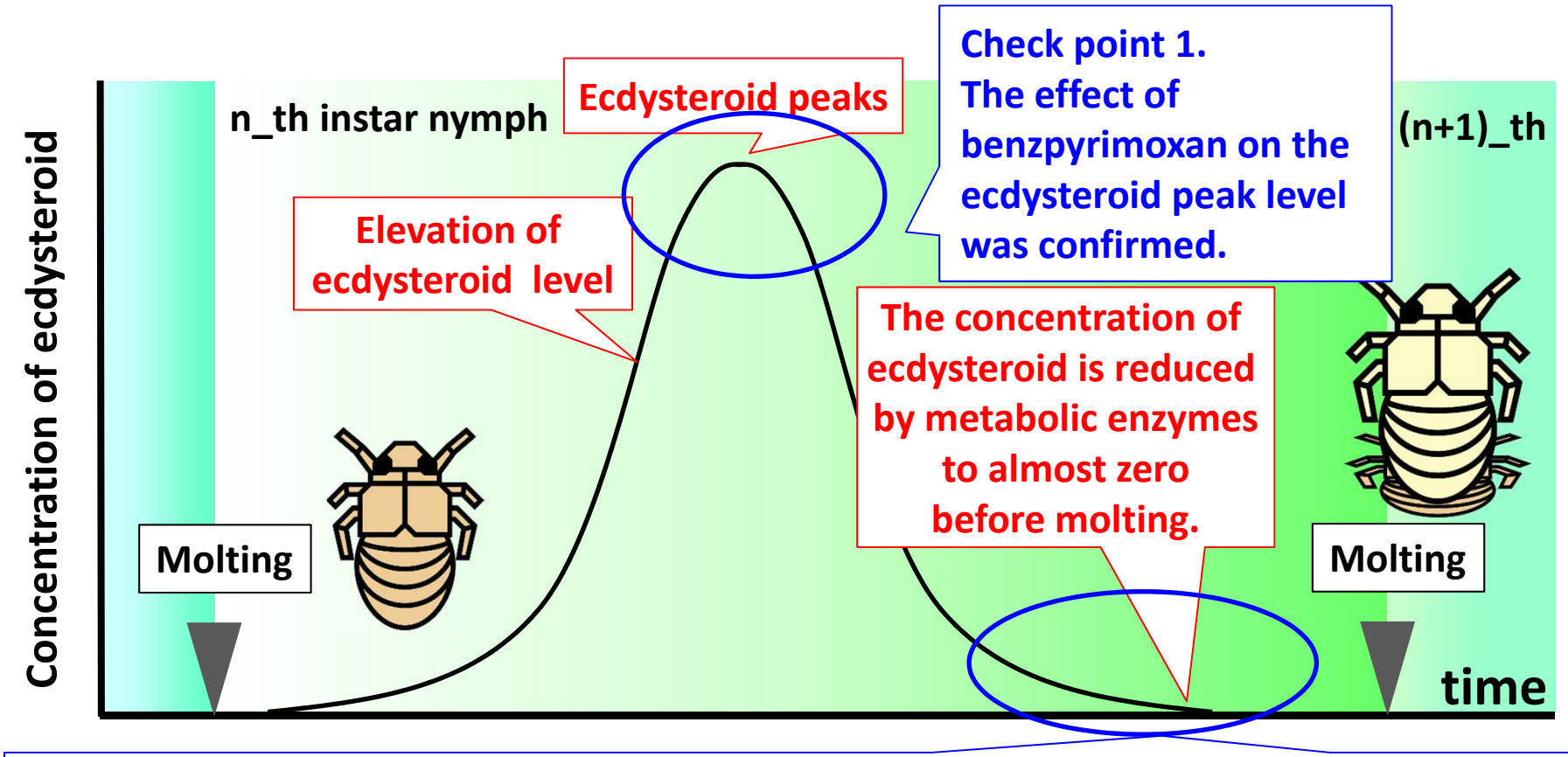
➡ It is also unlikely to affect juvenile hormone levels.

→ Does it affect the titer of molting hormone (ecdysteroids)?

Dynamics of the titer of ecdysteroids through molting phase



Dynamics of the titer of ecdysteroids through molting phase

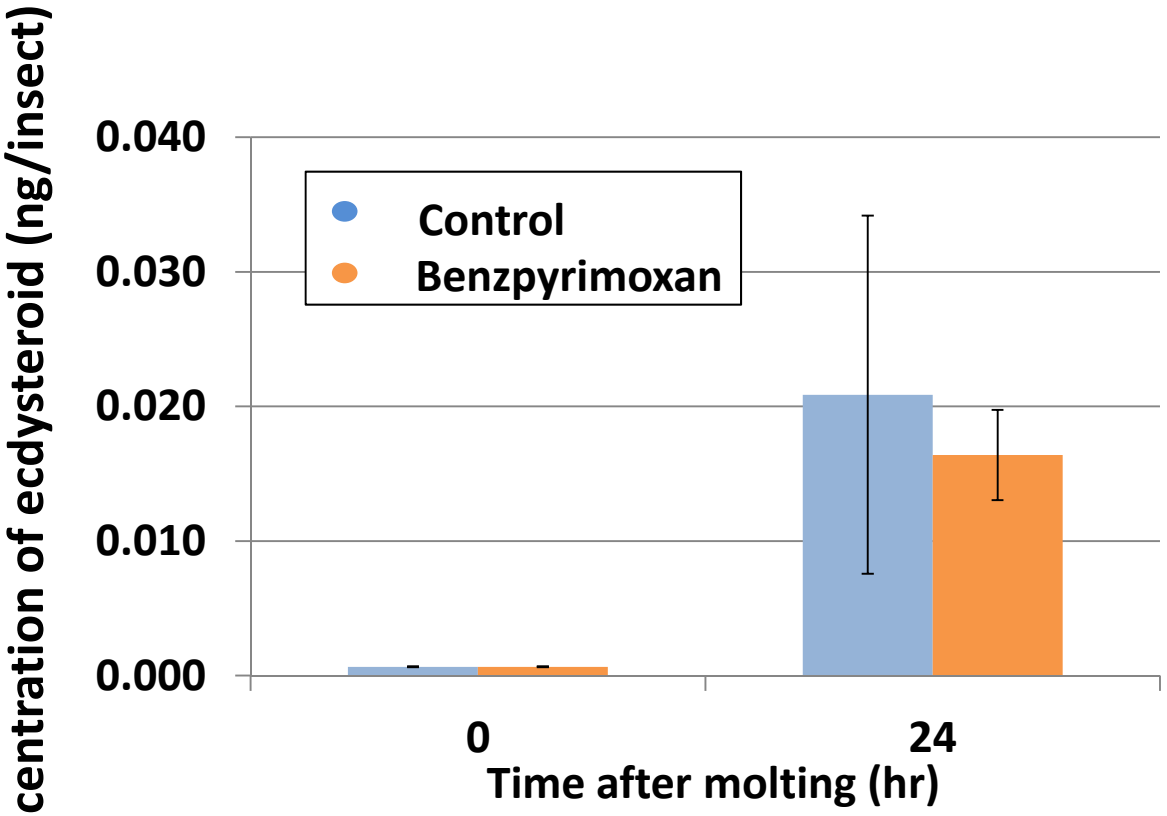


The ecdysteroid, which is reduced below a threshold level in normal condition, remains by benzpyrimoxan application. This may cause a disruption in the mechanism of molting.

Check point 2.

A time course study of the concentration of ecdysteroids was conducted in bph just before molting.

Check point 1: Effects of benzpyrimoxan on 20-hydroxyecdysone peak



It was reported that the peaks of 20-hydroxyecdysone in the fourth instar nymph of the brown planthopper are 24 / 33 hours after molting . (Pesticide Biochemistry & Physiology, 34, 9-16)

There was no significant difference in the peak levels of 20-hydroxyecdysone between the control and benzpyrimoxan applicated individuals.

Summary of methods

The fourth instar nymph were collected immediately after molting. (Wakayama strain)

↓

Nymphs were inoculated into rice plants treated with 100 ppm benzpyrimoxan.

↓

Insects were collected 0 and 24 hours after molting.

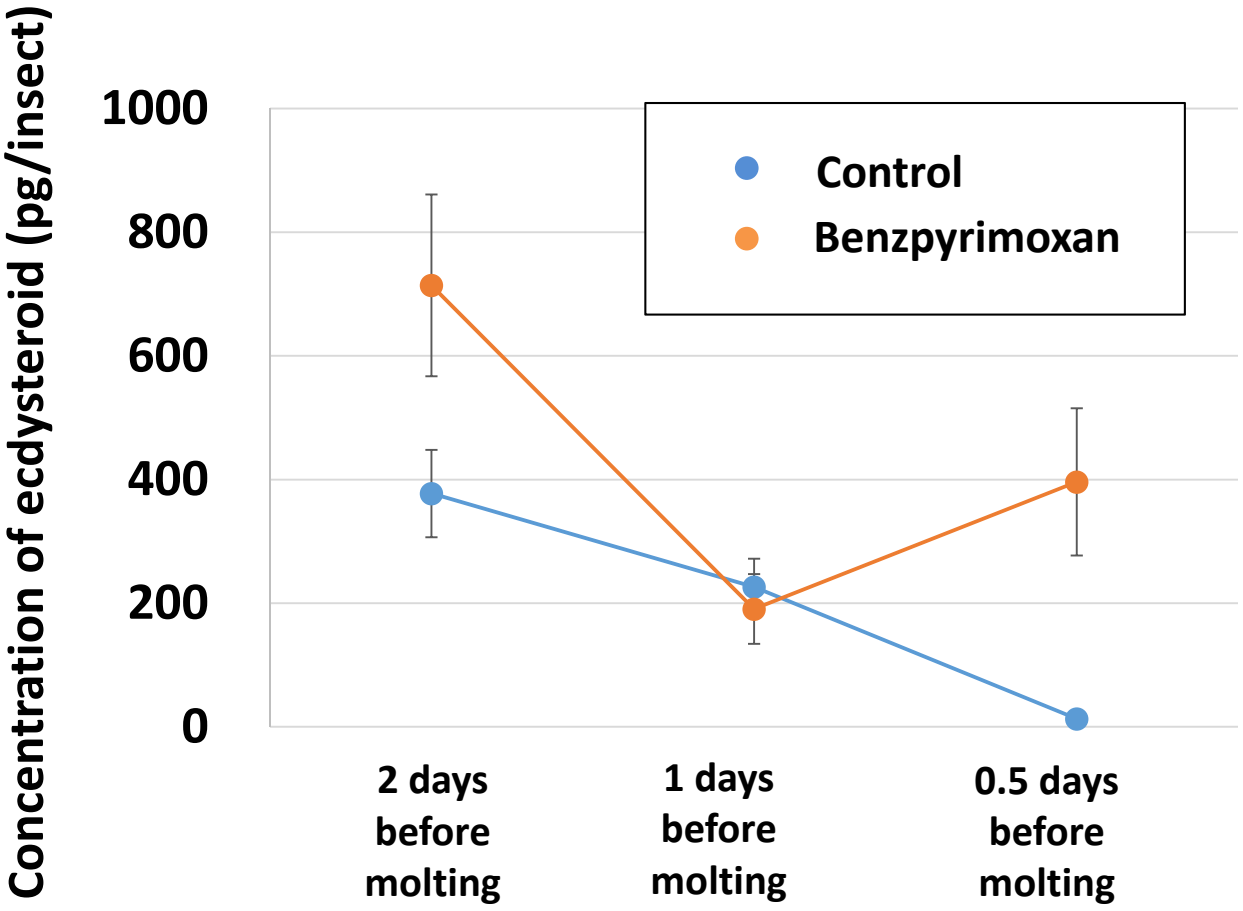
↓

Extracted with 80% MeOH

↓

20-hydroxyecdysone concentration was measuraed by LC/MS/MS (n = 3, Ave. & SD were represented)

Effects of benzpyrimoxan on ecdysteroids titer (BPH, Shimane strain)



In the control samples, the concentration of ecdysteroids decreased at 0.5 days before molting, whereas it remained at a relatively higher level in the applicated individuals.

Summary of methods

The fifth instar nymph were collected immediately after molting. (Shimane strain)

↓

Nymphs were aplicated topically with 0.083 µL of 0.001 % benzpyrimoxan acetone solution (around of LD_{50})

↓

Insects were collected 2, 1, and 0.5 days before molting calculated from the fifth instar nymph period (approximately 4 days).

↓

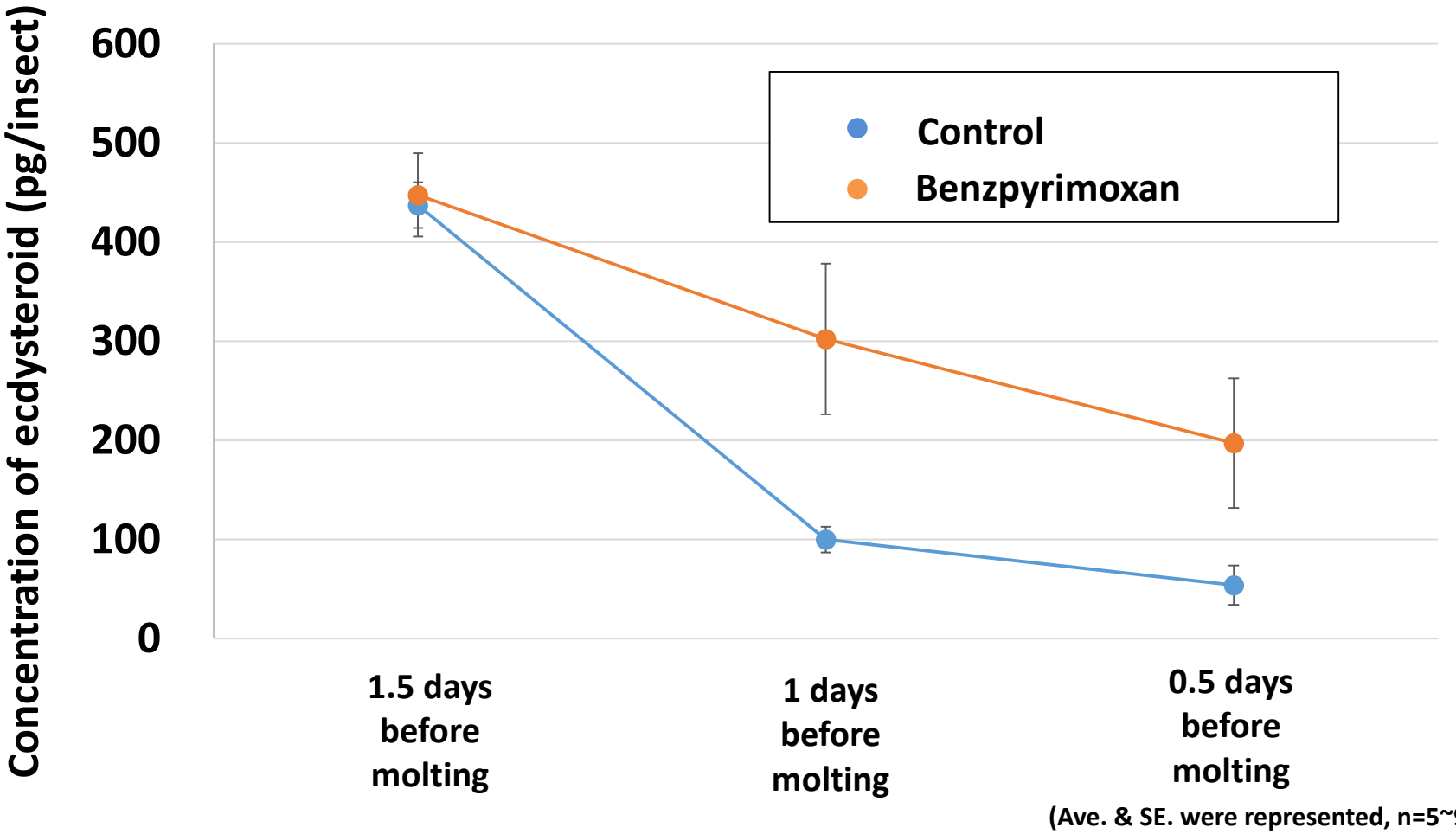
Extracted with methanol

↓

Ecdysteroids concentration was mesuraed by Enzyme Immuno Assay (n = 5 or 6, Ave. & SE were represented)

K. Matsukura, et al.: Abstr. 46th Annu. Meeting Pestic. Sci. Soc. Jpn., p. 126, 2021.

Effects of benzpyrimoxan on ecdysteroids titer (BPH, Wakayama strain)



(Ave. & SE. were represented, n=5~9)

The same phenomenon was observed in another strain. Suppression of ecdysteroids reduction by Benzpyrimoxan is strain-independent.

K. Matsukura, et al.: Abstr. 46th Annu. Meeting Pestic. Sci. Soc. Jpn., p. 126, 2021 (in Japanese).

Estimation of action mechanism from biological characteristics

●Suppression of ecdysteroids reduction by benzpyrimoxan was observed.

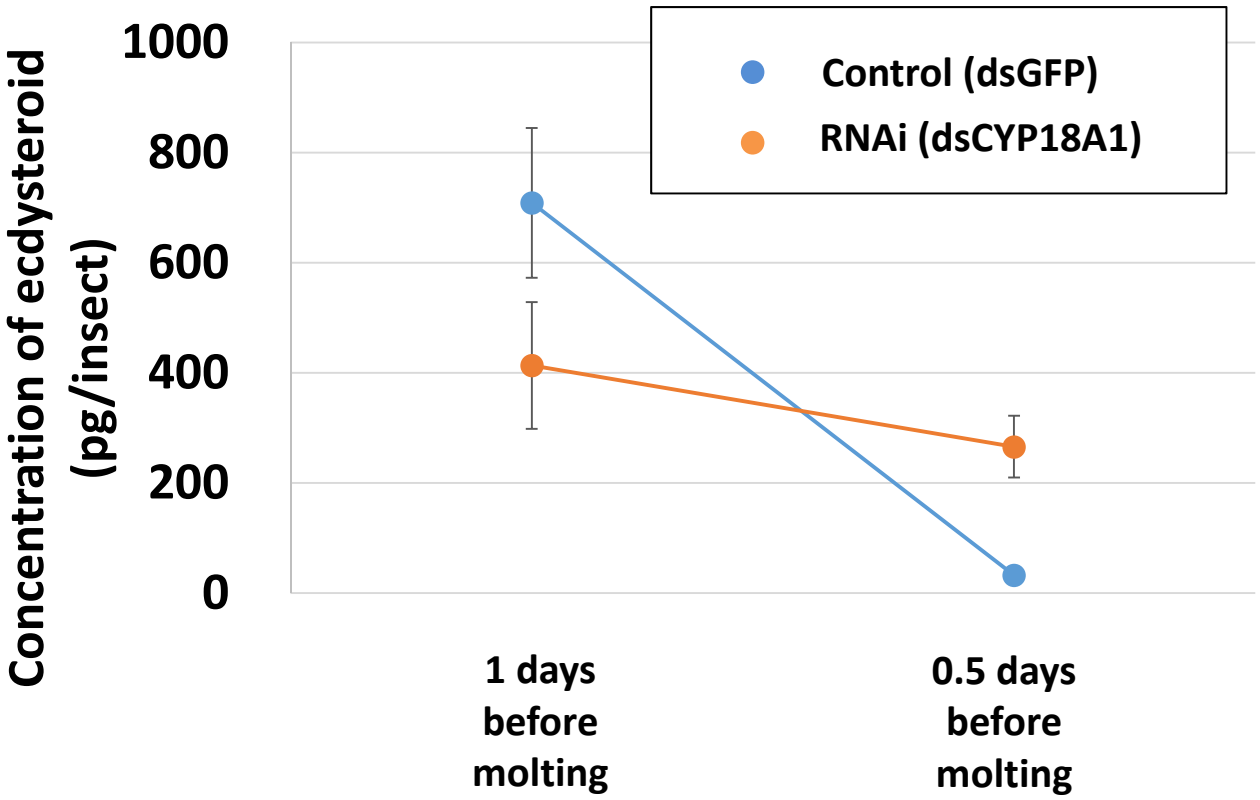
Does the benzpyrimoxan affect ecdysteroid metabolism?

Does the same phenomenon occur when the metabolism of ecdysteroids is actually suppressed?

Using RNAi method, we suppressed the expression of CYP18A1, a metabolic enzyme of ecdysteroid*, and confirmed its effect on the titer of ecdysteroids in BPH.

*Developmental Biology 349 (2011) 35–45

Effects of CYP18A1 RNAi on ecdysteroids titer (BPH, Shimane strain)



K. Matsukura, et al.: Abstr. 46th Annu. Meeting Pestic. Sci. Soc. Jpn., p. 126, 2021 (in Japanese).

The same method was used as in the benzpyrimoxan application study, except that RNAi was treated by injection.

(Ave. & SE. were represented、n=5~9)

In the control samples, the concentration of ecdysteroids decreased 0.5 days before molting, whereas it remained at relatively a higher level when CYP18A1 was suppressed by RNAi, similar to the benzpyrimoxan application.
In addition, some of the CYP18A1 RNAi-treated individuals showed abnormal molt symptoms.

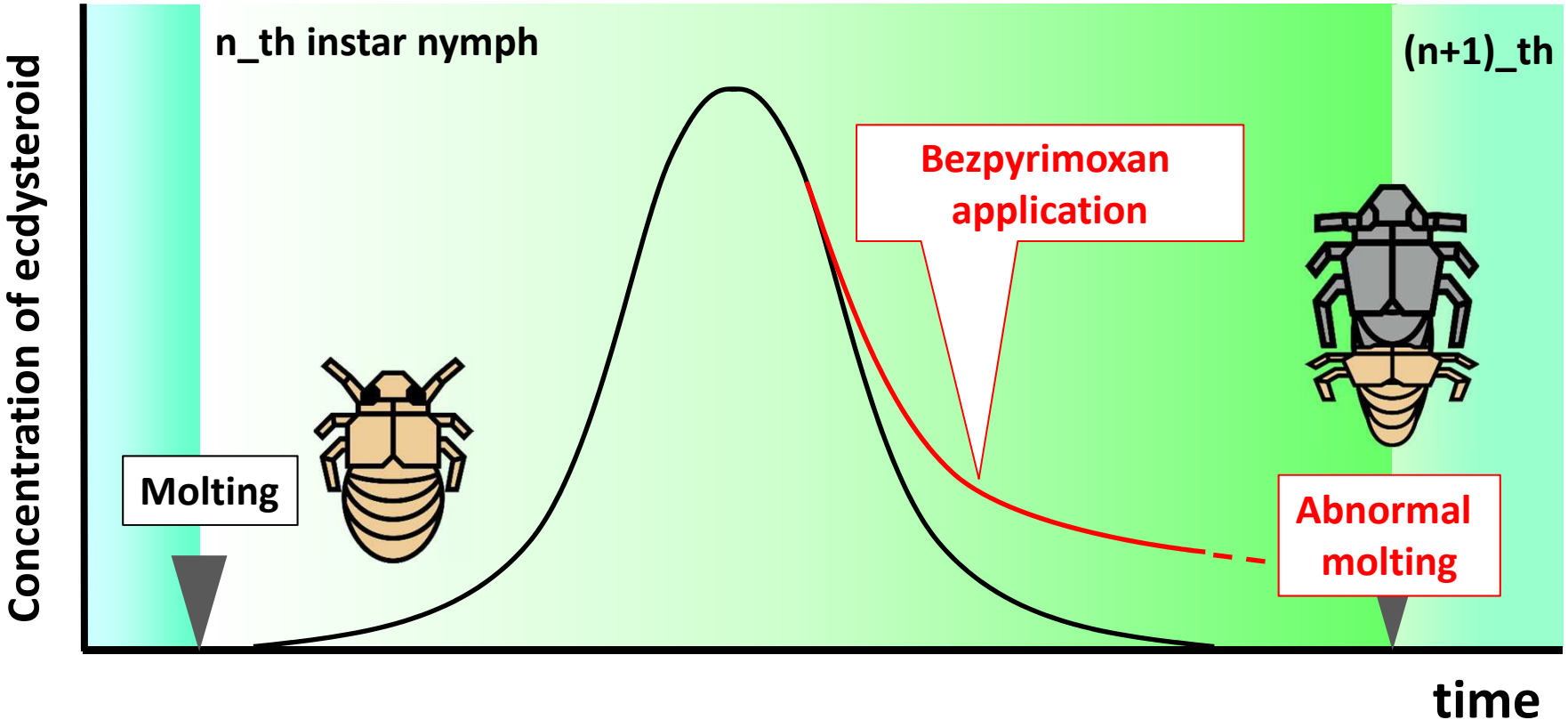
Summary of MoA study

- Benzpyrimoxan does not directly inhibit chitin biosynthesis, and the mechanisms of action of benzpyrimoxan and buprofezin are different.

- Suppression of ecdysteroids reduction was observed at benzpyrimoxan applicated individuals, suggesting an effect on metabolism.

- When CYP18A1, a metabolic enzyme of ecdysteroids, was suppressed by RNAi, ecdysteroids tended to remain in the hopper, which was similar to that observed with benzpyrimoxan application. In addition, the individuals knocked down by RNAi showed abnormal molting.

Effects of benzpyrimoxan on ecdysteroids titer (BPH)



These results suggest that the benzpyrimpxan induce abnormal molting by disrupting the titer of ecdysteroids in the planthopper, therefore, it have a novel mode of action different from that of existing molting inhibitors.

Cross resistance study on BPH (Japan)

Insecticides	LC_{50} (mg a.i./L)			
	Field collected strain (A)	Field collected strain (B)	Field collected strain (C)	Susceptible strain (D)
Benzpyrimoxan	0.33	0.32	0.23	0.12
Etofenprox (Group 3)	10	20	29	3.1
Imidacloprid (Group 4)	12	32	16	0.061
Fipronil (Group 2)	5.1	19	2.5	0.040
Buprofezin (Group 16)	21	5.3	12	0.27

Resistance ratio: ≤3　Resistance ratio: 3-30　Resistance ratio: >30

A:Nagasaki, 2020,　B:Kagoshima, 2019,　C:Kagoshima, 2015　D:Nichino strain, 1983

Insect pest: brown planthopper 3rd instar nymph / Application: dipping rice seedling / Investigation:5-7days after application

- Benzpyrimoxan showed high insecticidal activity against insusceptible populations of existing insecticides.

Cross resistance study on BPH (India)

Insecticides	Estimated LC_{90} (mg a.i./L)			
	Field collected strain (E)	Field collected strain (F)	Field collected strain (G)	Susceptible strain (D)
Benzpyrimoxan	0.3-1	0.1-0.3	0.3-1	0.1-0.3
Thiametoxam (Group 4)	3-10	10-30	1-3	0.03
Buprofezin (Group 16)	>300	30-100	10-30	0.3-1
Ethiprole (Group 2)	NT	10	30-100	<0.1

Resistance ratio: ≤3 | Resistance ratio: 3-30 | Resistance ratio: >30

E:Karnataka, 2018, F:Telangana, 2017, G:Andhra Pradesh, 2015 D:Nichino strain, 1983 (Japan)

Insect pest: brown planthopper 3rd instar nymph / Application: dipping rice seedling / Investigation:5-7days after application

- Benzpyrimoxan showed high insecticidal activity against insusceptible populations of existing insecticides.

Proposal

New Group “Ecdysone titer disrupters” is requested

- Benzpyrimoxan belongs to a novel chemical class as “benzyloxypyrimidines”.
- Benzpyrimoxan is considered to cause abnormal molting by disrupting the titer of ecdysteroids in the planthopper, and have a novel mode of action different from that of existing molting inhibitors.
- No cross-resistance to existing insecticides (Japan and India strains).