IPRRG presentation

Elevated CO_2 may alter pheromonal communication in *Helicoverpa armigera*

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2018.10.16

RURAL DEVELOPMENT ADMINISTRATION
NATIONAL INSTITUTE OF HORTICULTURAL AND HERBAL SCIENCE

Physiological Entomology

Physiological Entomology (2018) **43**, 169–179 DOI: 10.1111/phen.12239

Elevated CO_2 may alter pheromonal communication in *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract. Carbon dioxide (CO_2) as a greenhouse gas has been increasing in recent decades. Because an elevated atmospheric CO_2 influences insect physiology and behaviour, we hypothesize that pheromone–mediated communication in the moth is affected by an increased CO_2 level. We test the behavioural responses of male *Helicoverpa armigera* to sex pheromone in a wind tunnel, demonstrating a significant reduction of approaching behaviour to the odour source at a high CO_2 level (1000 ppm). Electroantennogram (EAG) responses of male to the pheromone component are also significantly suppressed in high CO_2 environments (600 and 1000 ppm), indicating that a high CO_2 level inhibits both behavioural and electrophysiological responses of male to the sex pheromone. Interestingly, the EAG response of the whole head preparation of males is influenced more by the elevated CO_2 level than that of the antenna-cut preparation. A sequential increase of CO_2 levels from an ambient CO_2 level also decreases the EAG response of the whole head but not of the labial palp-removed head, implying a potential mediation of labial palp in the head where the CO_2 receptor is located. By contrast, sex pheromone production in females reared under or shifted to an elevated CO_2 condition is increased, and the putative underlying mechanism for this is discussed. The present study provides an insight into the adaptive strategy of moth pheromone communication in a changing environment.

Key words. Carbon dioxide, electroantennogram, *Helicoverpa armigera*, pheromone, wind tunnel.

Introduction

Carbon dioxide (CO_2) is a greenhouse gas that plays a significant role in global warming. The atmospheric concentration of CO_2 has dramatically increased in recent decades and is predicted to reach 538–936 ppm by the end of this century (IPCC, 2013). Understanding how rising atmospheric CO_2 levels affect insect communication is important for predicting potential changes in the physiology and behaviour of insect pests and the damages that they cause. Several studies report the effects of elevated CO_2 on chemical communication in insects (Boullis *et al.*, 2016). For example, increased CO_2 levels reduce the ability of a moth (*Cactoblastis catorum*) to detect host plants (Stange, 1997) and the escape behaviour associated with alarm pheromone is diminished under elevated CO_2 in the aphids *Chaitophorus stevensis* (Mondor *et al.*, 2004), *Sitobion avenae* (Sun *et al.*, 2010) and *Amphorophora idaei* (Hentley *et al.*, 2014). Also, an increase in CO_2 concentration reduces the production and emission of alarm pheromone in the aphid species *Acyrthosiphon pisum* (Boullis *et al.*, 2017). Because pheromones play a crucial role in mating, aggregation and alarm signals in insects (Rutowski, 1982; Cardé

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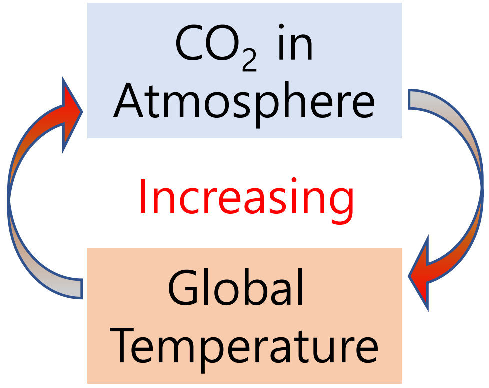
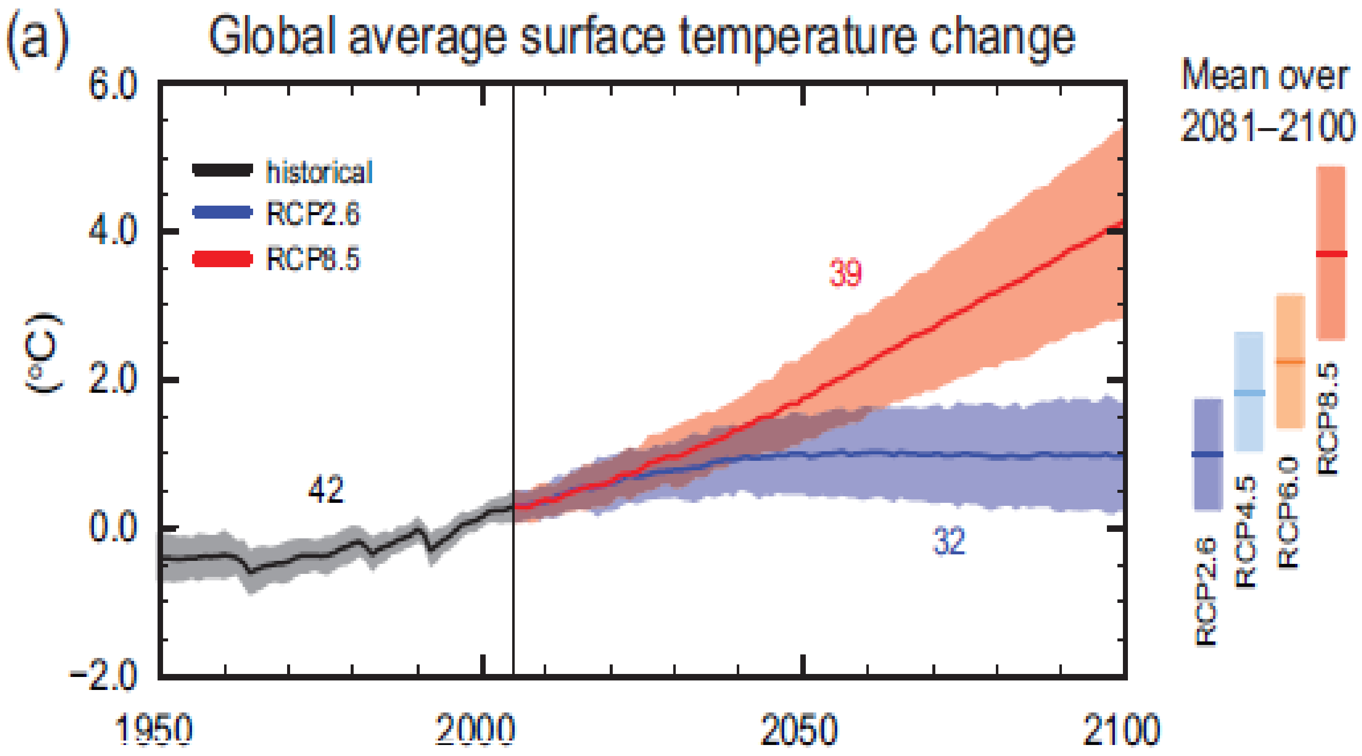
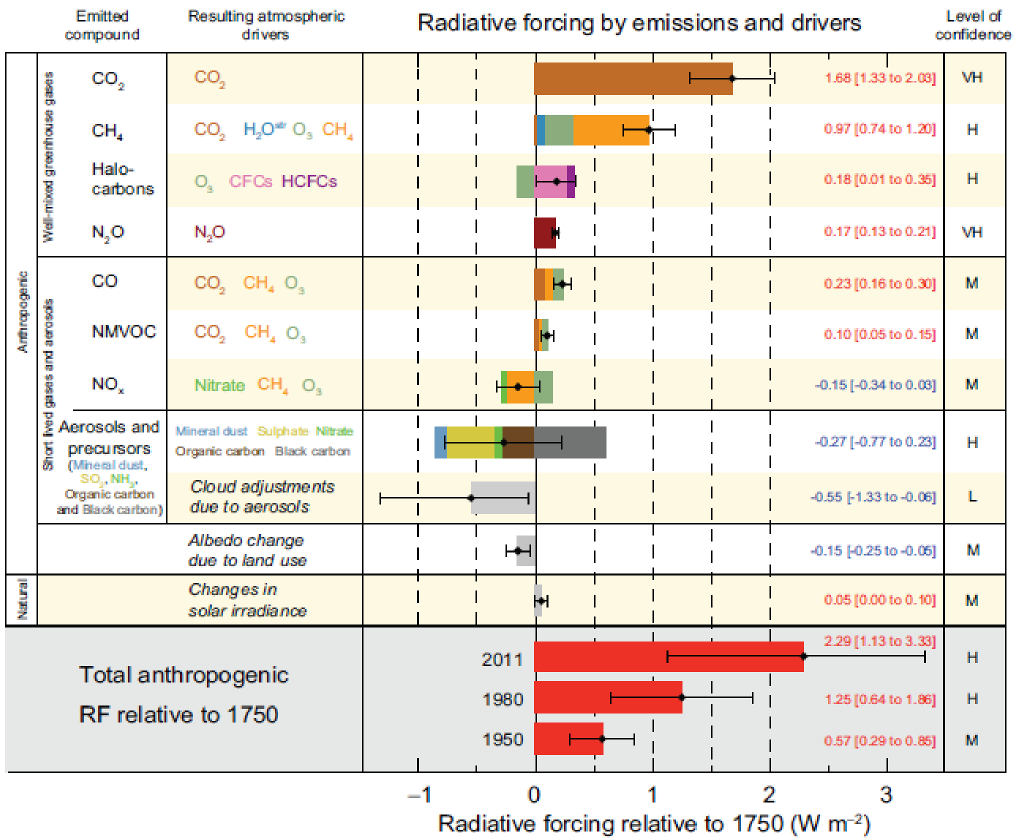
I. INTRODUCTION

1. Climate change

Recent global warming was caused by Green house gases(GHG) from fossil fuel utilization and CO_2 gas in atmosphere is the major driving force of global warming according to 5th report of IPCC

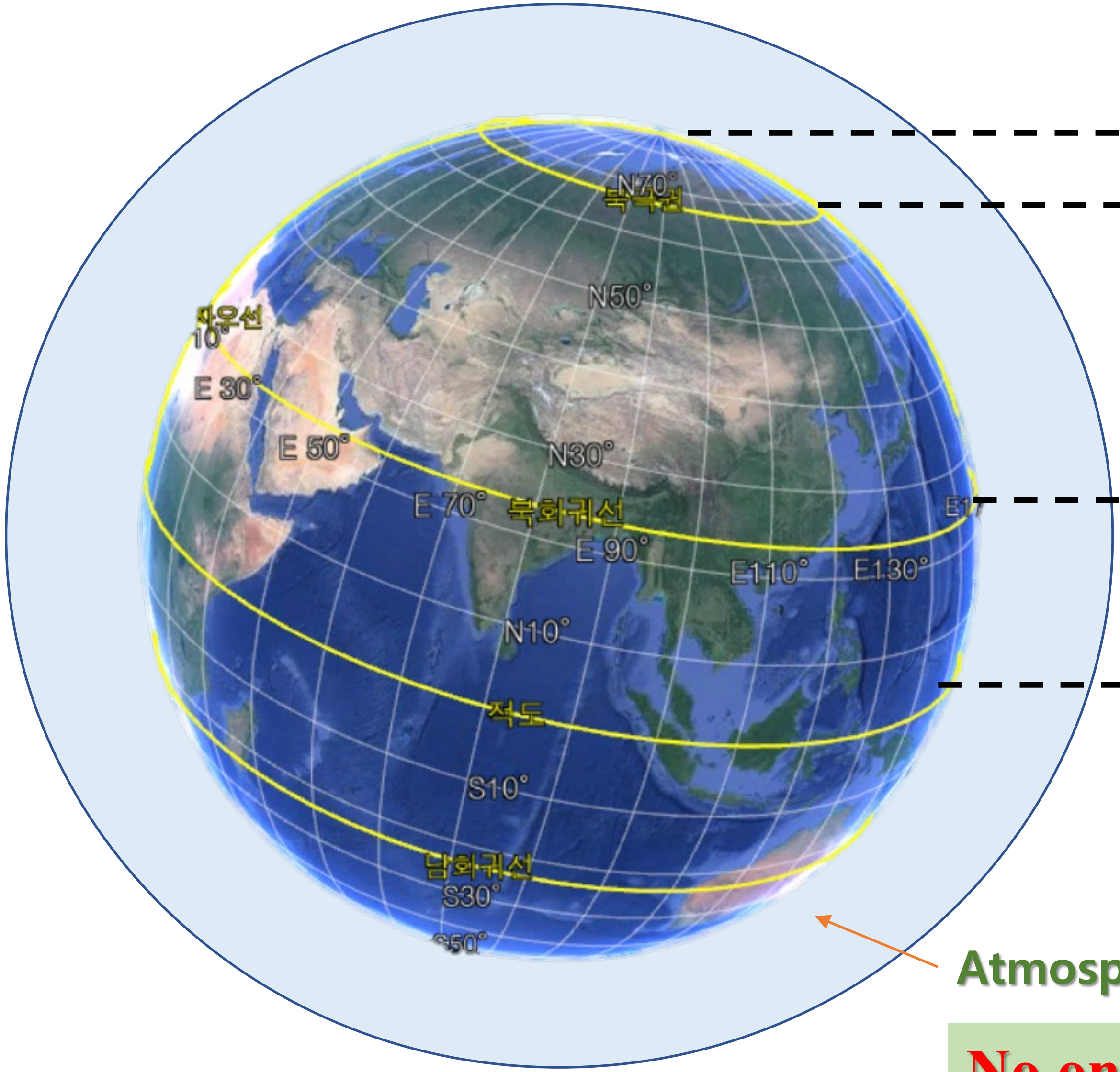


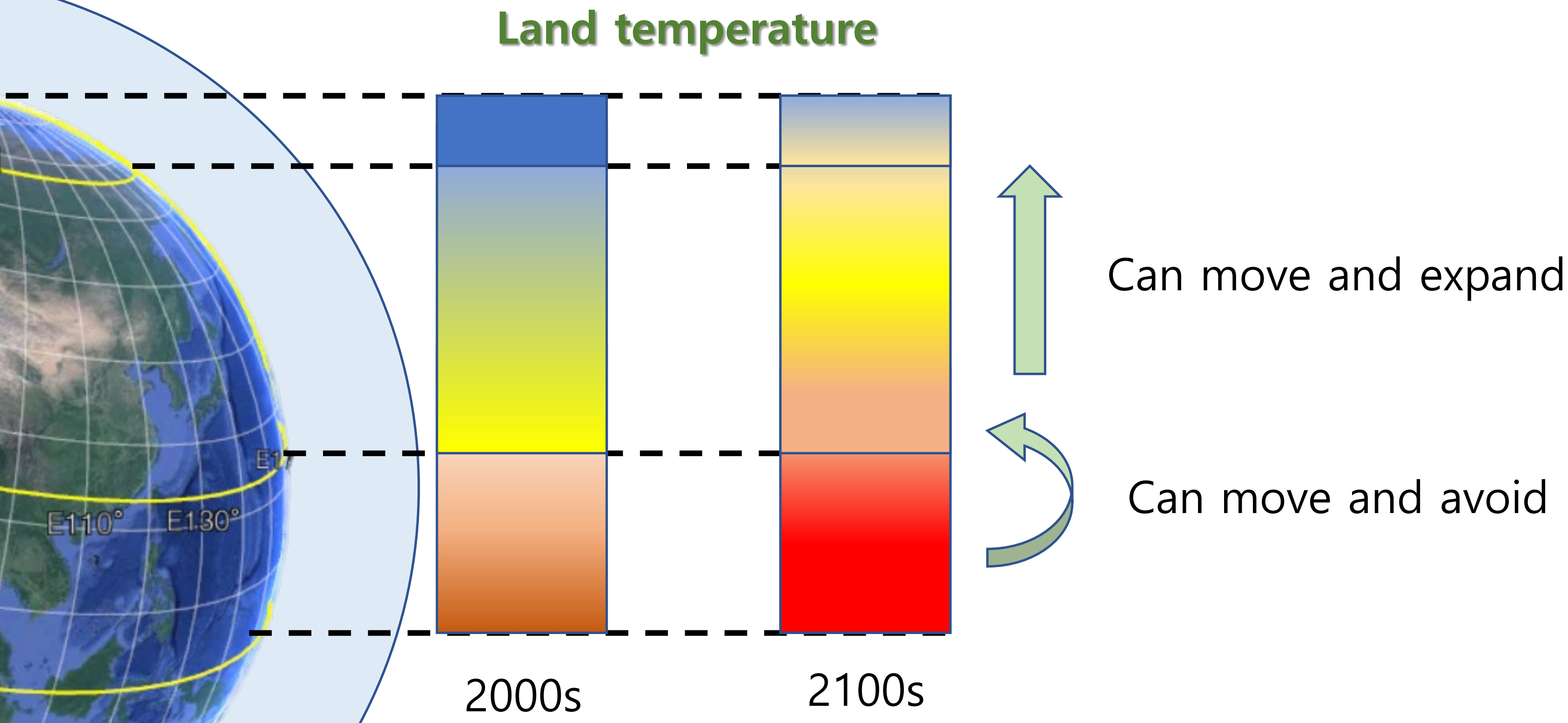




- Figure from 5th report of IPCC

Q1. Which is more crucial threating factor caused by global warming? Temperature or CO_2





Atmosphere : CO_2 concentration is almost same in the world

No one on the earth can run away from CO_2 trap

- Earth from the google earth

2. CO_2 level in Future

CO_2 gas concentration in atmosphere is expected to reach 538 to 936 ppm by 2100.

* RCP2.6 and RCP4.5 is too ideal so that it would be 600~1,000 ppm

Table AII.4.1: CO_2 abundance (ppm)

Year	Observed	RCP2.6	RCP4.5	RCP6.0	RCP8.5	A2	B1	IS92a	Min	RCP8.5[&]	Max
PI	278 ± 2	278	278	278	278	278	278	278			
2011[obs]	390.5 ± 0.3										
2000		368.9	368.9	368.9	368.9	368	368	368			
2005		378.8	378.8	378.8	378.8					378.8	
2010		389.3	389.1	389.1	389.3	388	387	388	366	394	413
2020		412.1	411.1	409.4	415.8	416	411	414	386	425	449
2030		430.8	435.0	428.9	448.8	448	434	442	412	461	496
2040		440.2	460.8	450.7	489.4	486	460	472	443	504	555
2050		442.7	486.5	477.7	540.5	527	485	504	482	559	627
2060		441.7	508.9	510.6	603.5	574	506	538	530	625	713
2070		437.5	524.3	549.8	677.1	628	522	575	588	703	810
2080		431.6	531.1	594.3	758.2	690	534	615	651	790	914
2090		426.0	533.7	635.6	844.8	762	542	662	722	885	1026
2100		420.9	538.4	669.7	935.9	846	544	713	794	985 ± 97	1142

Notes:
For observations (2011[obs]) see Chapter 2; and for projections see Box 1.1 (Figure 2), Sections 6.4.3.1, 11.3.1.1, 11.3.5.1.1. RCPn.n refers to values taken directly from the published RCP scenarios using the MAGICC model (Meinshausen et al., 2011a; 2011b). These are harmonized to match observations up to 2005 (378.8 ppm) and project future abundances thereafter. RCP8.5[&] shows the average and assessed 90% confidence interval for year 2100, plus the

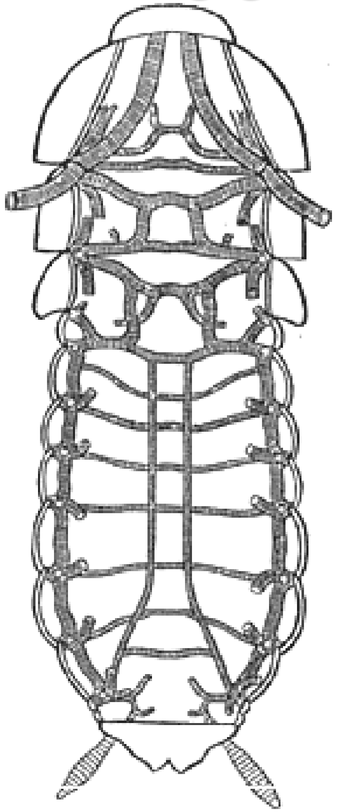
- **Table from 5th report of IPCC**

Q2. Can such slight increase in CO_2 concentration in atmosphere affect insect?

No crucial direct-effect on Animals on the land was reported at a range of such CO_2 level.

Some studies on the interaction between insects and host plant can be summarized as

Chewing group has negative effect while sucking group has no or positive effect.



- 600~1,000 ppm = 0.06 ~ 0.1 %
- 1% = 10,000 ppm

- During respiration

  - CO_2 remains 3~5 % in tracheal system

- Even if ambient CO_2 level increase 600~ 1000 ppm, it must be much lower than that in inner of insect. However, such sensor organs as porous receptor in antenna can be affected

※ A few papers discussed that such range of conc. of CO_2 gas can directly affect the CNS in the inside of insect <= Right or Wrong?

- Figure from google's image list

※ Intoxication of CO_2 gas

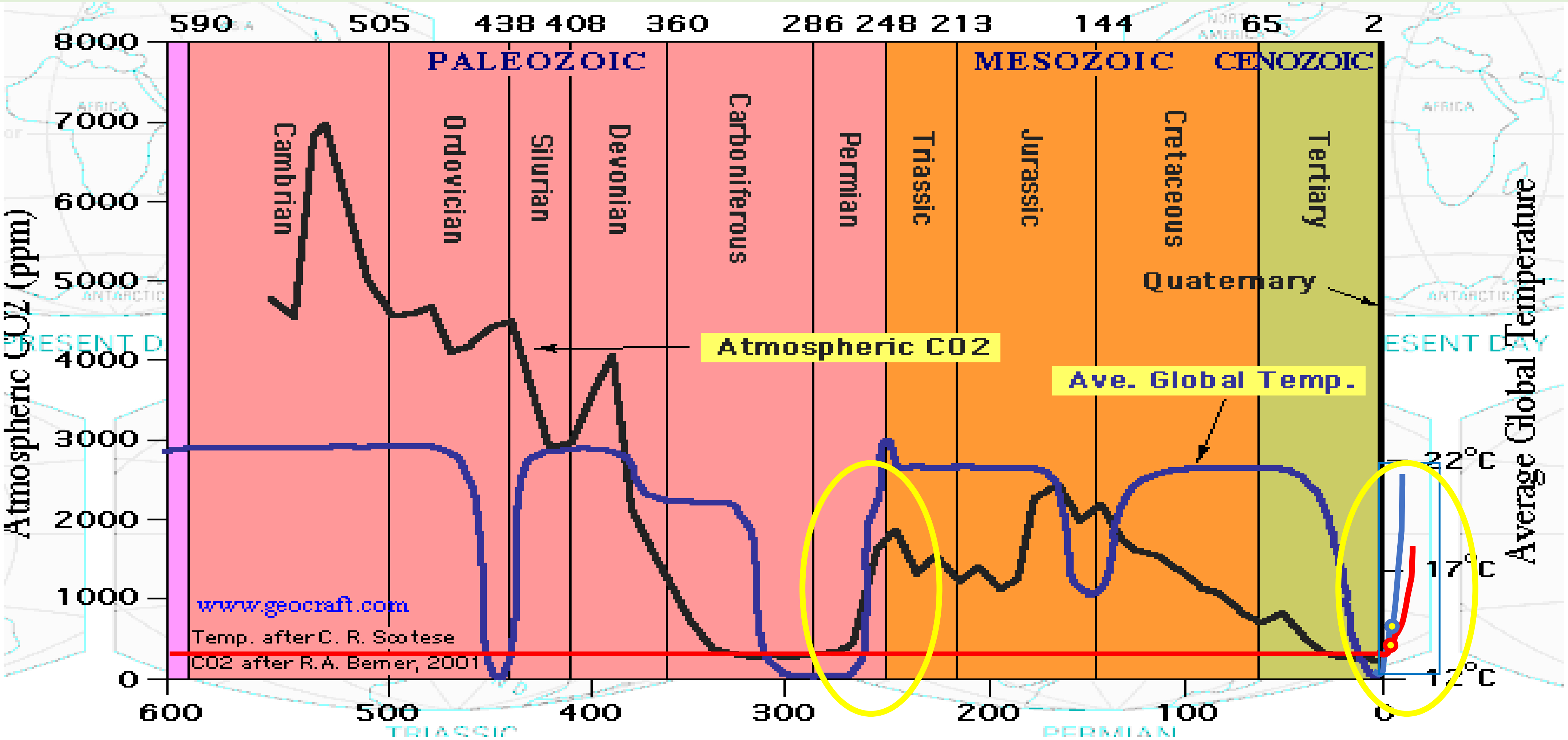
High con. of CO_2 gas cause a cardiac arrest and a physiological abnormality in Insect and also very risky to human in a closed environment.

1 to 1.5%	Slight effect on chemical metabolism after exposures of several hours.	**Longer exposure can be risky to human**
3%	The gas is weakly narcotic at this level, giving rise to deeper breathing, reduced hearing ability, coupled with headache, an increase in blood pressure and pulse rate.	**Highly Risky to human; be lethal without avoidance**
4 - 5%	Stimulation of the respiratory centre occurs resulting in deeper and more rapid breathing. Signs of intoxication will become evident after 30 minutes exposure.	
5 - 10%	Breathing becomes more laborious with headache and loss of judgement.	**Lethal to human; promptly can lose consciousness without awareness and go to die**
10 -100%	When the carbon dioxide concentration increases above 10%, unconsciousness will occur in under one minute and unless prompt action is taken, further exposure to high levels will eventually result in death.	

- Document from a MSDS of CO_2 gas

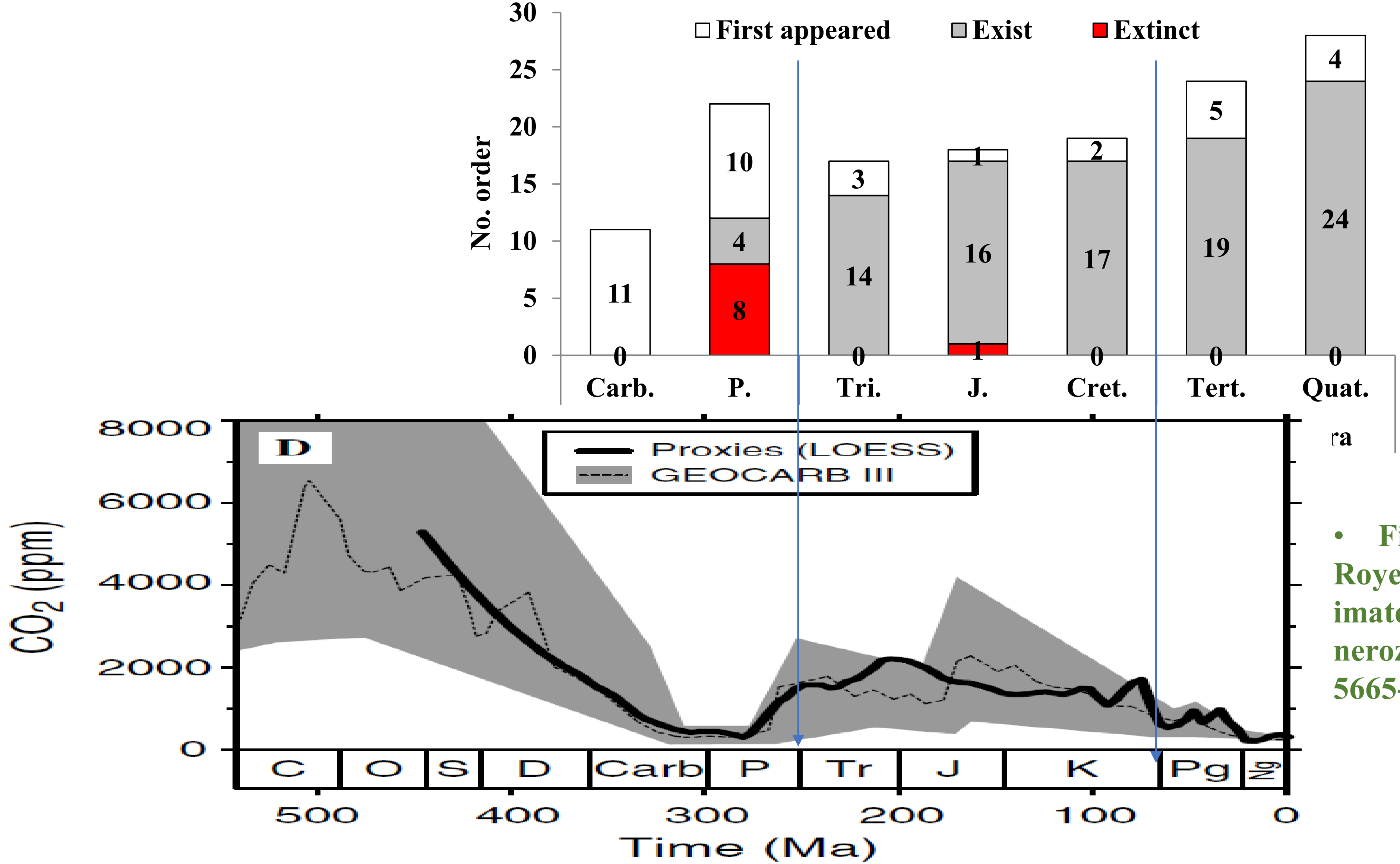
3. CO_2 level in atmosphere in the past of the earth

- CO_2 gas concentration increasement : 300 to 2,000 ppm in Permian and to 2,500 ppm in Jurassic
- In the end of Permian, 97% organism in ocean was disappeared and also 40% of insects did.



Q3. Is such increase in CO_2 level a factor that cause the insect's extinction in the past?

Extinction of Insect seems to be coincident with the increase in CO_2 concentration

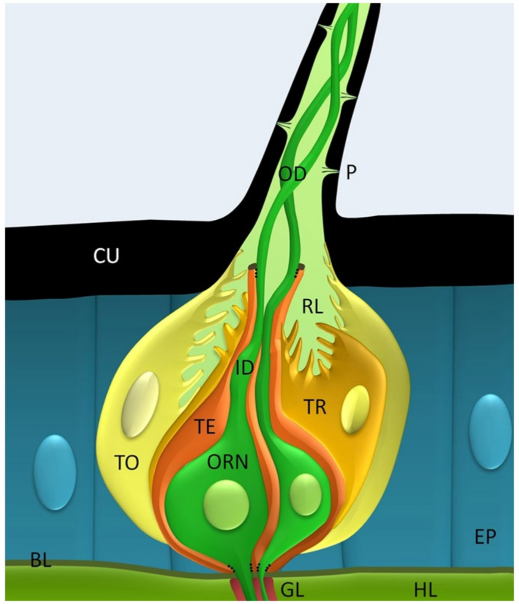


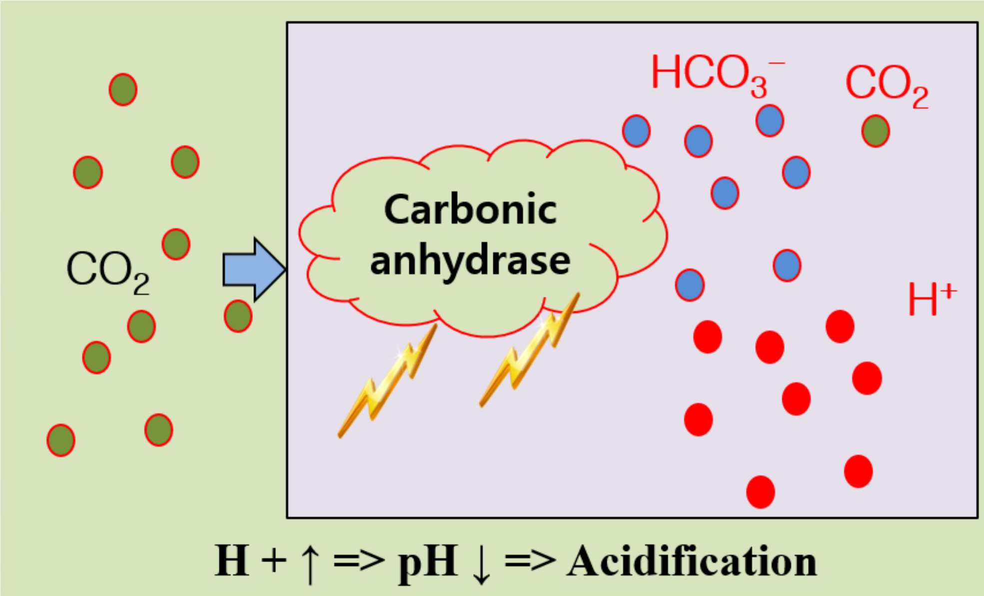
- Data collected from : Carpenter, F. M., Burnham, L. 1985. The geological record of insects. Ann. Rev. Earth Pl. Sci. 13: 297-314.

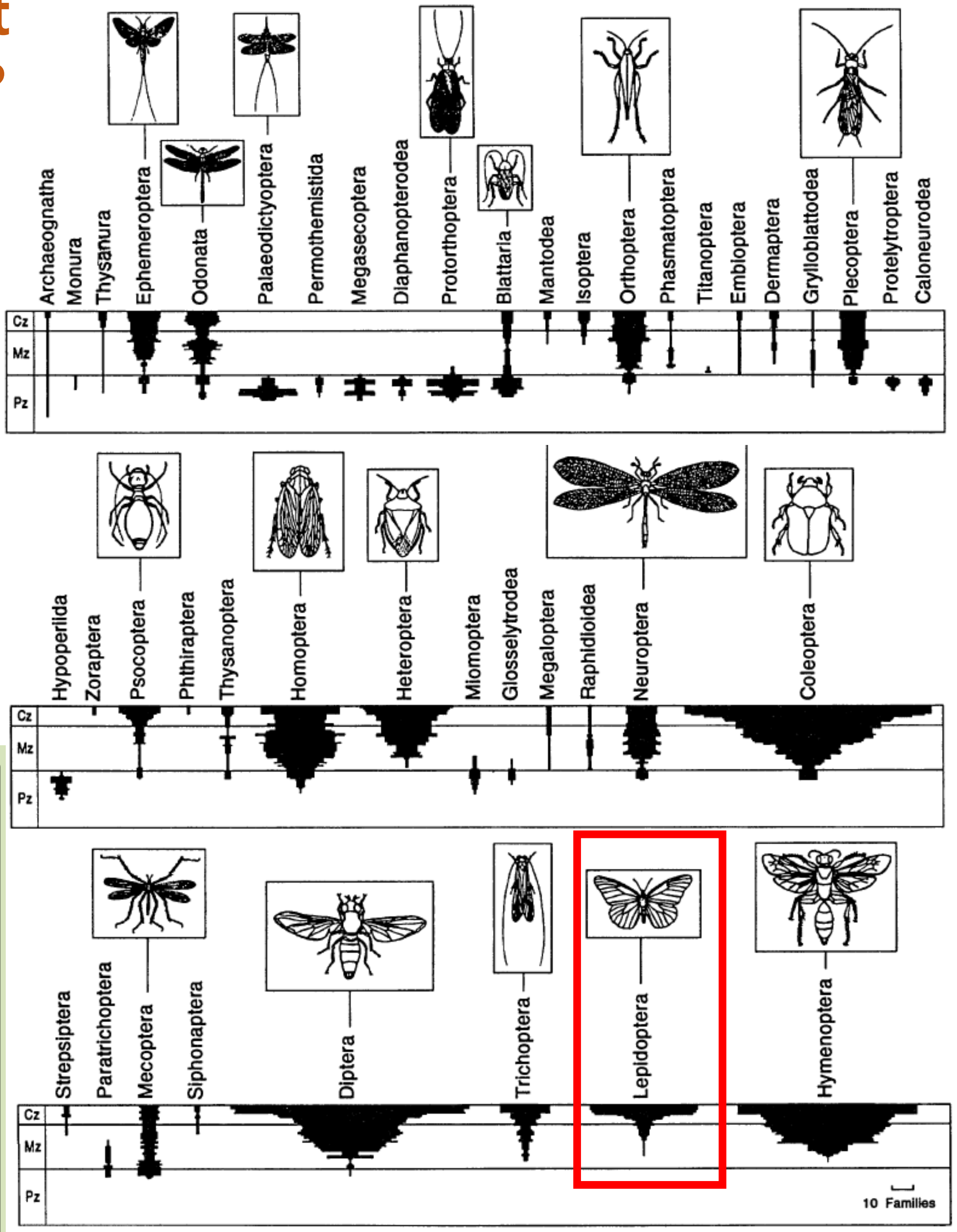
- Figure from : Royer, D. L. 2005. CO2-forced cl imate thresholds during the pha nerozoic. Geo. Cosmo. Acta. 70: 5665-5675.

Q4. Could the increased CO_2 concentration affect the sex pheromone communication in a moth?

- Lepidoptera species evolved mostly in Cenozoic when CO_2 level is below 500 ppm.
- Moths live independently on ground and they use sex pheromones as a long distance communication for mating.
- Sex pheromone receptors in antenna are a porous structure with own lymph where CO2 gas easily infiltrate and can affect their function.







* Figure from : Labandeira, C. C., Sepkoski, J. J. 2005. Insect diversity in the fossil record. Science. 261:310-315.

4. Summary of a previously conducted experiment (unpublished)

- Response of Male's antennae to sex pheromone decreased as CO_2 level increased, However, it looks like that **sex pheromone production of female increased and was prolonged.**

=> an interspecies complementary system between male and female?

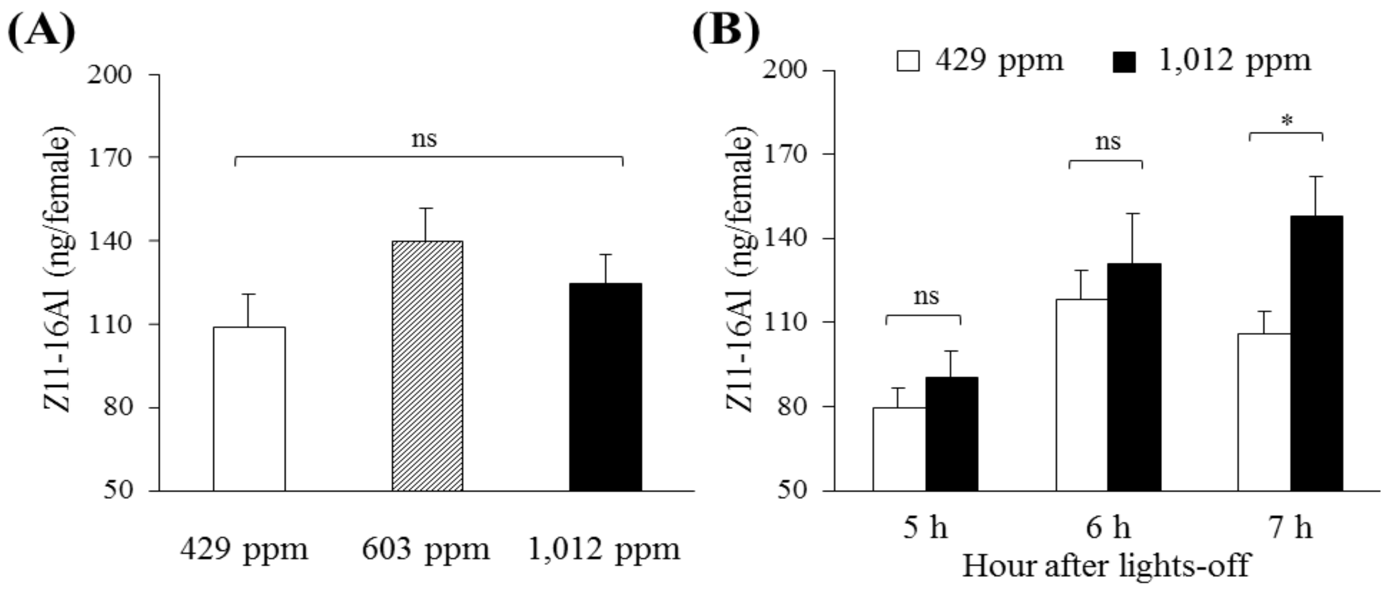


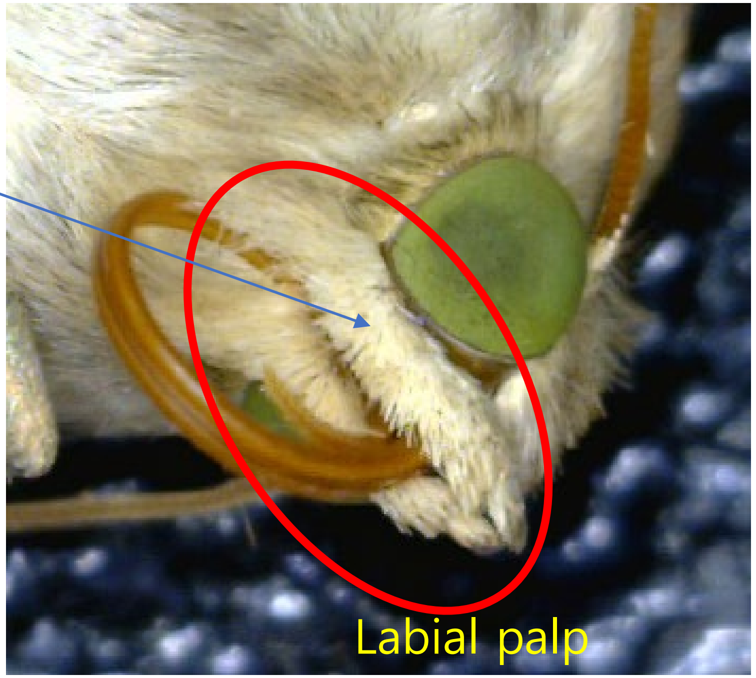
Fig. 3 Sex pheromone production of *H. armigera* females (2 d old) in different CO_2 levels of 429 ppm, 603 ppm, and 1,012 ppm where adults were reared from egg. (A) Average amounts of a major sex pheromone component, *Z*11-16Al, produced per female at 6 h after lights-off were 108.9 ± 11.54 ng (N = 15) in 429 ppm, 139.8 ± 11.62 ng (N = 33) in 603 ppm, and 124.6 ± 10.39 ng (N = 23) in 1,012 ppm; ns, not significantly different by HST test at P = 0.05, (B) Average amounts of *Z*11-16Al produced per female at 5 h, 6h, and 7 h after lights-off were 79.4 ± 7.15 ng (N = 11), 118.1 ± 10.75 (N = 20), and 106.0 ± 7.72 ng (N = 22) in 429 ppm and 90.5 ± 8.63 ng (N = 13), 130.9 ± 18.45 (N = 20), and 148.1 ± 14.04 ng (N = 20) in 1,012 ppm. Student's t-tests at P = 0.05: ns, not significantly different; *, significantly different.

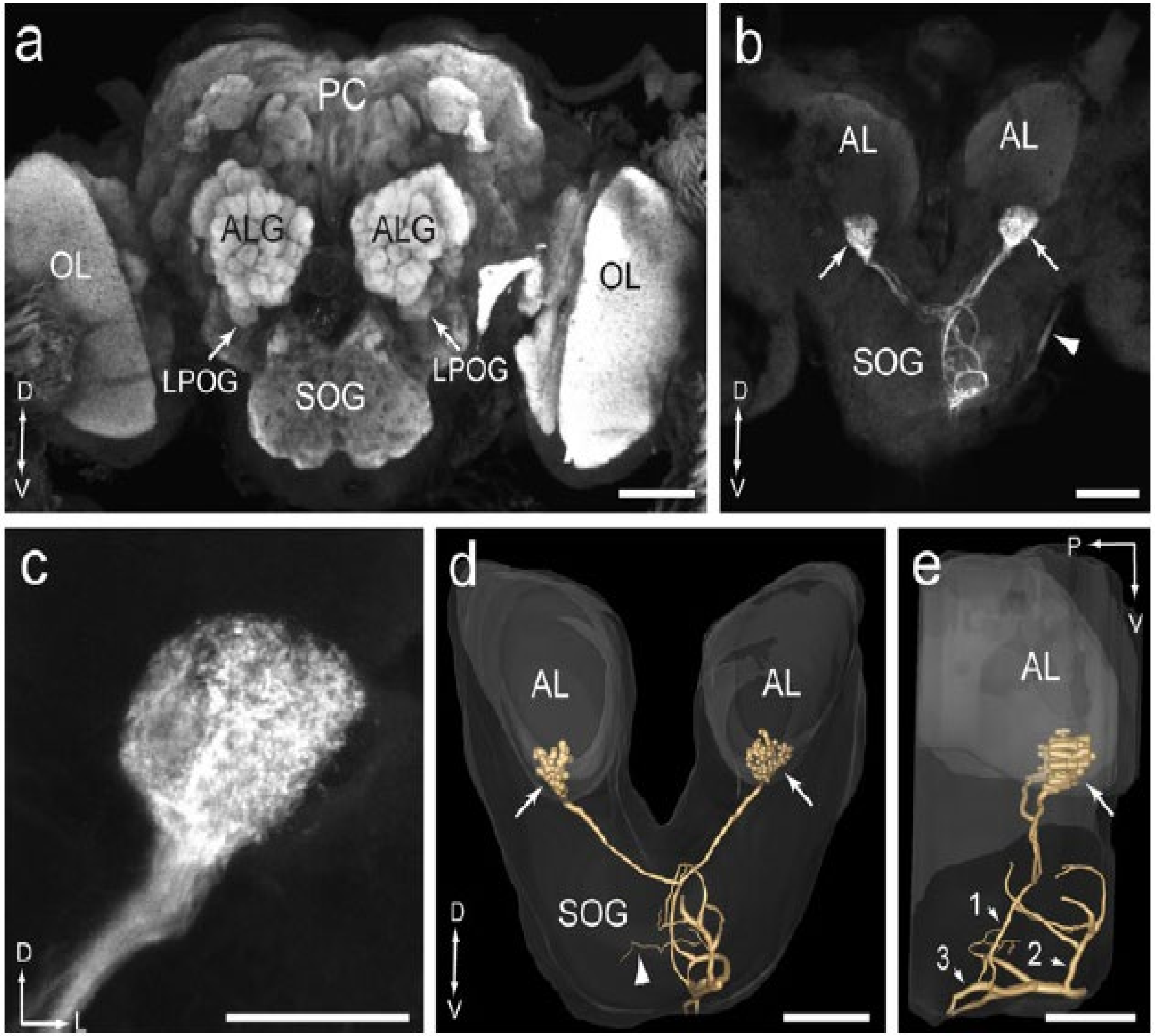
- **Q5. How the female control the pheromone production as for the outside of CO_2 level?**

4. CO_2 receptor in labial palps

- Moth has CO_2 receptor in their labial palps but their role is not known well.
- CO_2 receptor projects to Antenna Lobe(AL), Suboesophageal Ganglion (SOG), Ventral Nerve Cord (VNC)

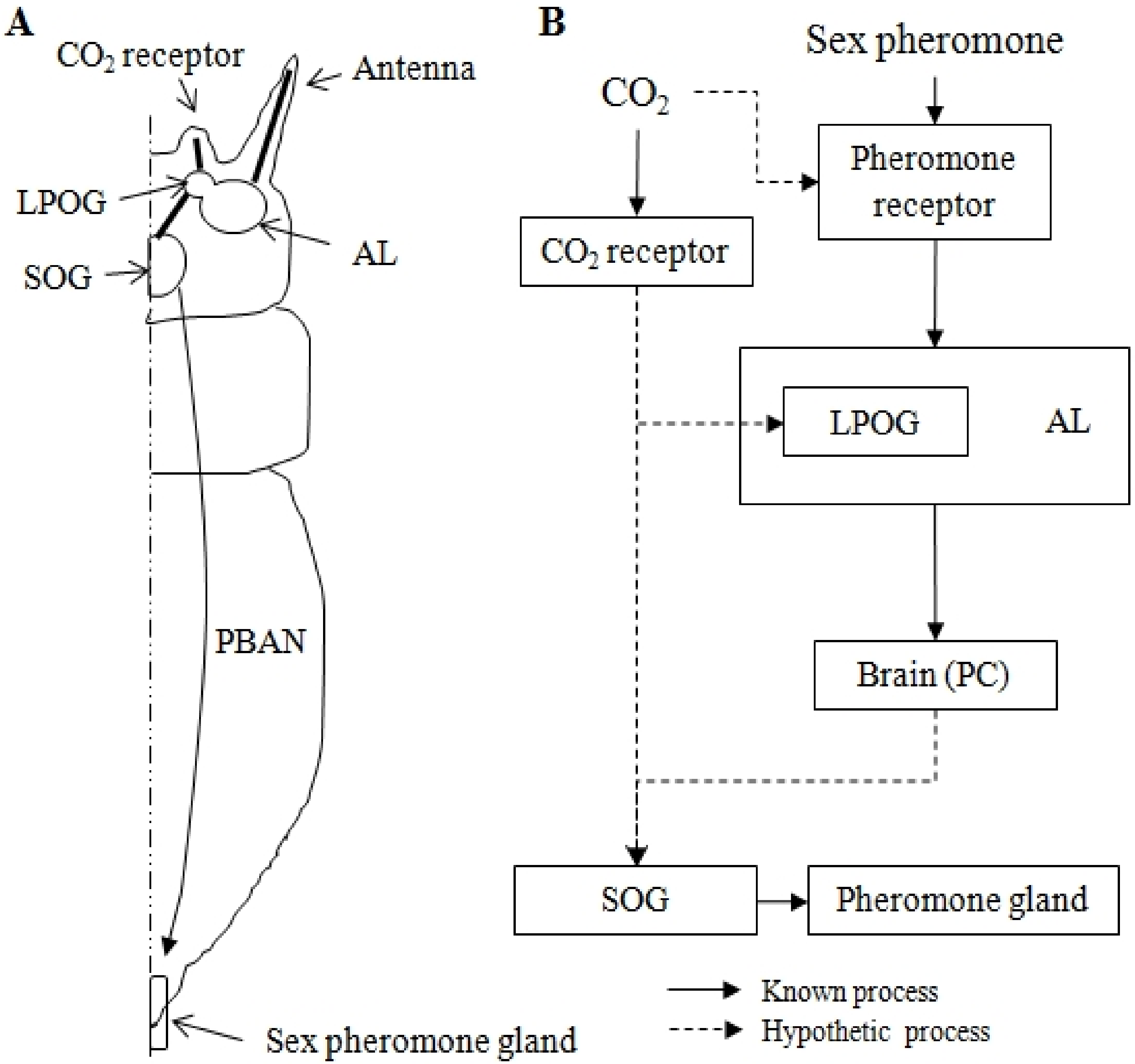
CO_2 receptor
Is In the labial palp





* Figure from Zhao et al(2013)

II. Hypothesis



< for male >

H1 : CO_2 directly affect the pheromone receptor and their signal intensity in male's antenna
=> changes in EAG (electroantennogram)

H2 : Signal from CO_2 receptor affect signal from antenna in Male's PC
=> changes in behavioral response

< for female >

H3 : Signal from CO_2 receptor affect SOG where PBAN is stored and released
=> changes in sex pheromone production

< for all : unpublished>

H4 : Signal from CO_2 receptor affect the heart
=> changes in the rate of heart beat.
(respiration is related)

H5 : CO_2 directly affect all chemical sensor
=> changes in finding food and digestion

=> Can affect the development and/or survival

III. Method and Results

1. CO_2 chambers

- **Room size : 2 m * 2 m * 2 m**
- **Treatment :**
  - Control : 400 ppm, CO_2 Treat. : 600, 1000 ppm
- **Insect : *Helicoverpa armigera* reared with an artificial diet and tested for 3 generations**

Table. Daily average values (Mean± STD)

Room	Temp	CO_2	RH	O_2
1000	**25 ± 0.1**	**965 ± 13.5**	**52± 1.8**	**21 ± 0.2**
600	**25 ± 0.1**	**604 ± 9.6**	**52 ± 1.7**	**21 ± 0.1**
400	**25 ± 0.1**	**451 ± 16**	**52 ± 1.8**	**21 ± 0.1**

- **Period : 2015.1.1.~5.19. * Data are collected with 1 h interval (3rd Experiment)**

1. Wind tunnel test

H 1,2 : Is there any change in male's behavioral response to the pheromone under various CO_2 level?

- **Odor source : 1 mg of sex pheromone lure (97.5:2.5 (Z11-16Al vs Z9-16Al))**
- **Sample : two group (Normal male, Labial palps removed male)**
- **Treatment : 400, 600, 1000 ppm of CO_2**
- **Examined behavior : Upwind, Approach, contact to lure for 100 males per CO2 treatment per Group**

Normal male

Labial palps removed male

* Male behavior to the sex pheromone in the wind tunnel device used in this study

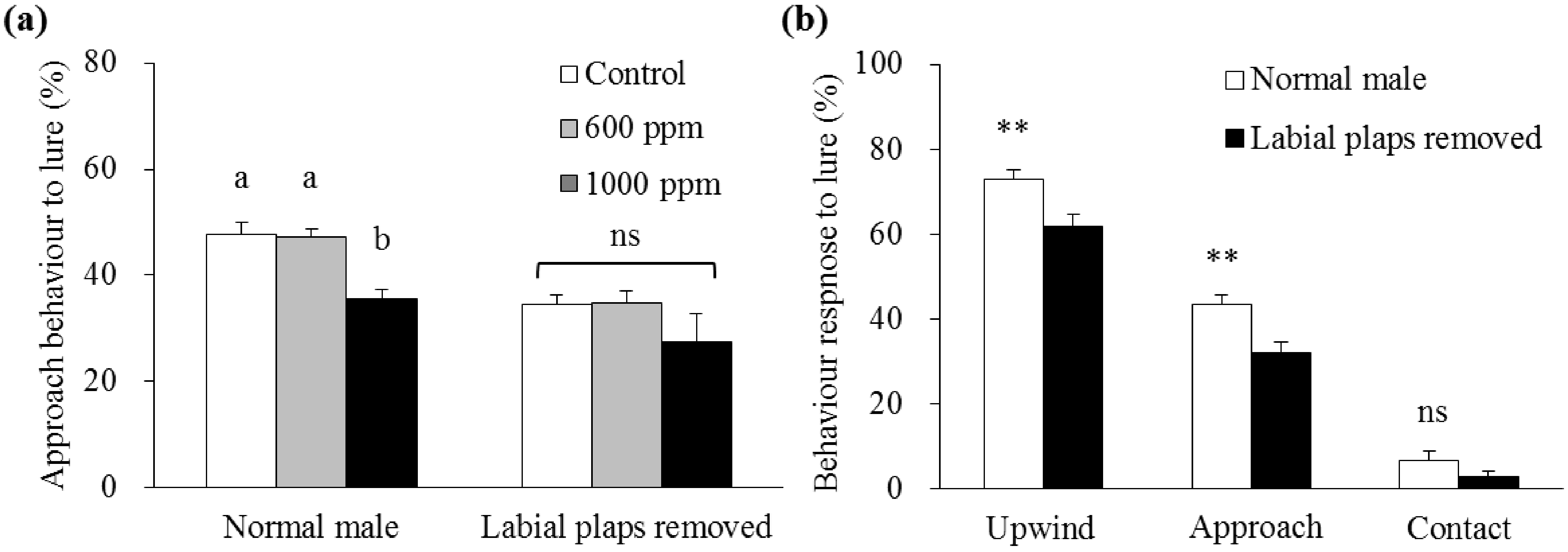


Figure 1. Wind-tunnel behavioural responses of *Helicoverpa armigera* males reared at different CO_2 levels. Data are presented as means ± standard error (SE). (A) Approach behaviour of males to 1 mg of sex pheromone lure containing *Z*11-16Al and *Z*9-16Al at a 97.5:2.5 ratio (normal males: n = 237, control; n = 246, 600 ppm; n = 228, 1,000 ppm; $P < 0.05$, labial palps removed: n = 215, control; n = 229, 600 ppm; n = 221, 1,000 ppm; $P > 0.05$). (B) Comparison of upwind, approach, and contact behavioural responses between normal males (n = 711) and those with the labial palps removed (n = 665) (: $P < 0.01$).**

- **Male showed reduced attractancy under higher CO_2 level => Perception ability may be affected**
- **Labial palps removed group showed less attractancy => CO_2 receptor may affect the decision**

2. EAG test1 : for the male adapted at each CO_2 level

H1 : Is there any change in male's antennal response to the pheromones among the CO_2 levels?

- **Odor source: Control(Air, hexane), Treatment (0.001, 0.01, 0.1, 1, and 10 ug of Z11-16 Al)**
- **Samples : males are reared at each 400, 600, and 1000 ppm of CO_2 level.**
- **EAG test: head (antenna +head), antenna**

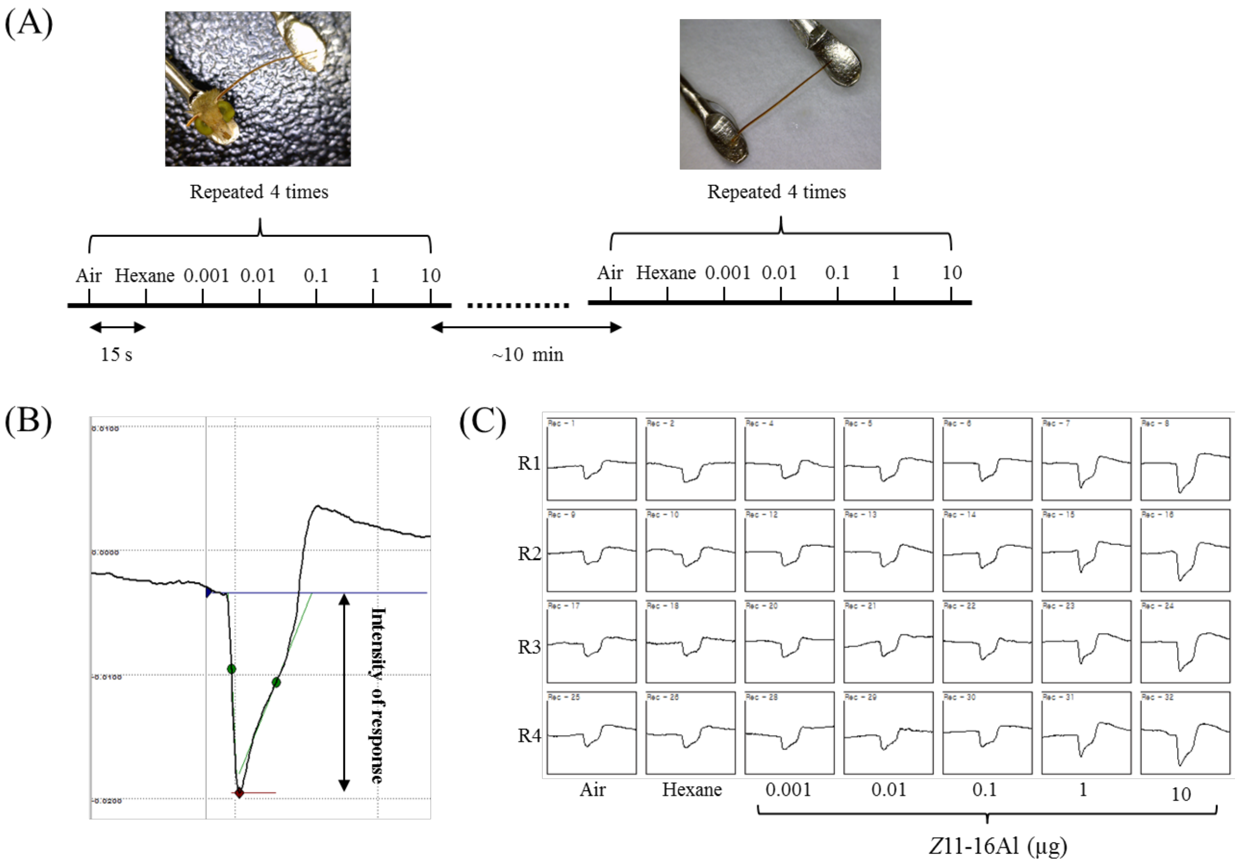


Figure S1. Electroantennogram (EAG) experiment on male antenna and head samples. (A) EAG process for population reared at CO_2 levels of 450 (control), 600, and 1,000 ppm, (B) Representative EAG response and intensity measurement, and (C) Representative EAG responses for an antenna at 450 ppm CO_2.

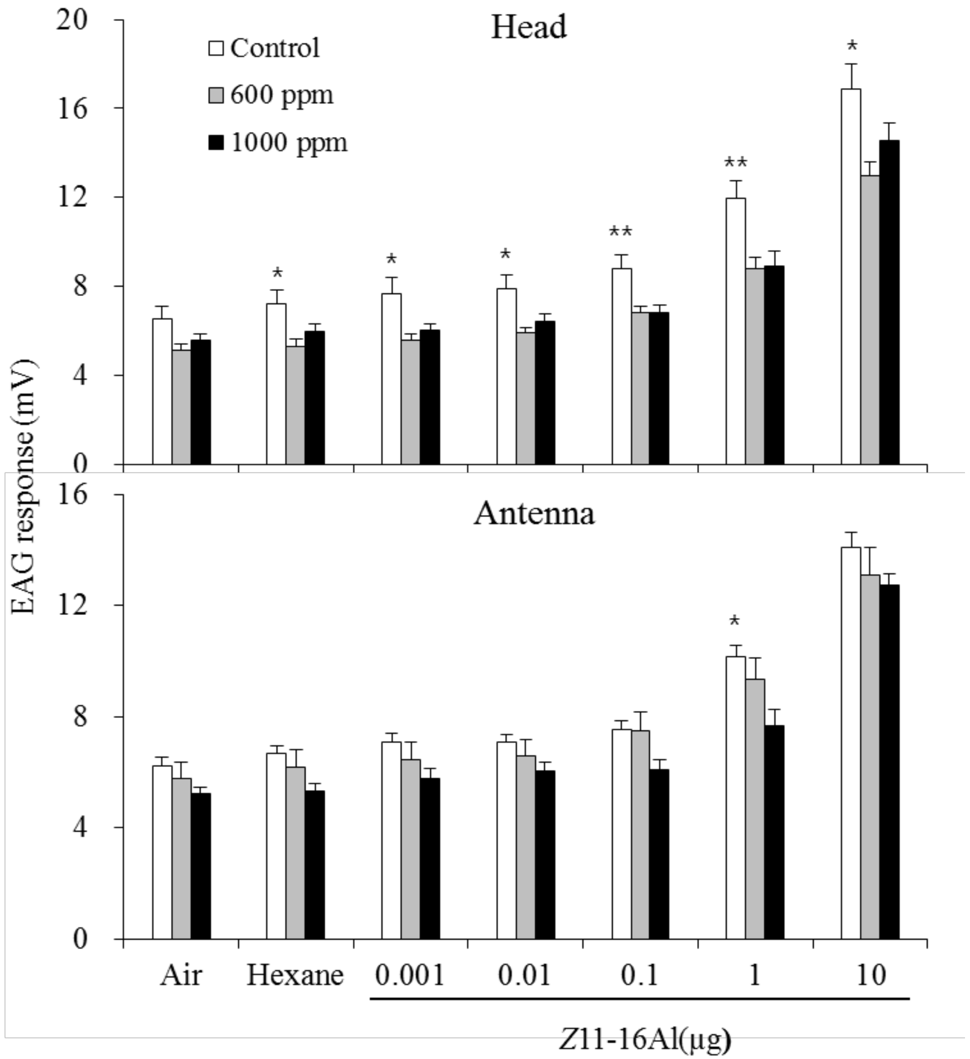


Figure 2. Electroantennogram (EAG) response of *H. armigera* males (age: 3 d) reared at different CO_2 levels to different concentrations of a synthetic sex pheromone component, *Z*11-16Al. EAG response of the head and antenna in turn (n = 10). Data are presented as means ± SE (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$).

- **Males adapted at higher CO2 level showed decreased EAG response.** **=> CO_2 directly affect antenna**
- **There is differences in EAG responses between antenna only and antenna+head.**

=> CO_2 receptor in labial palps may have a role in interpretation of signal from antenna

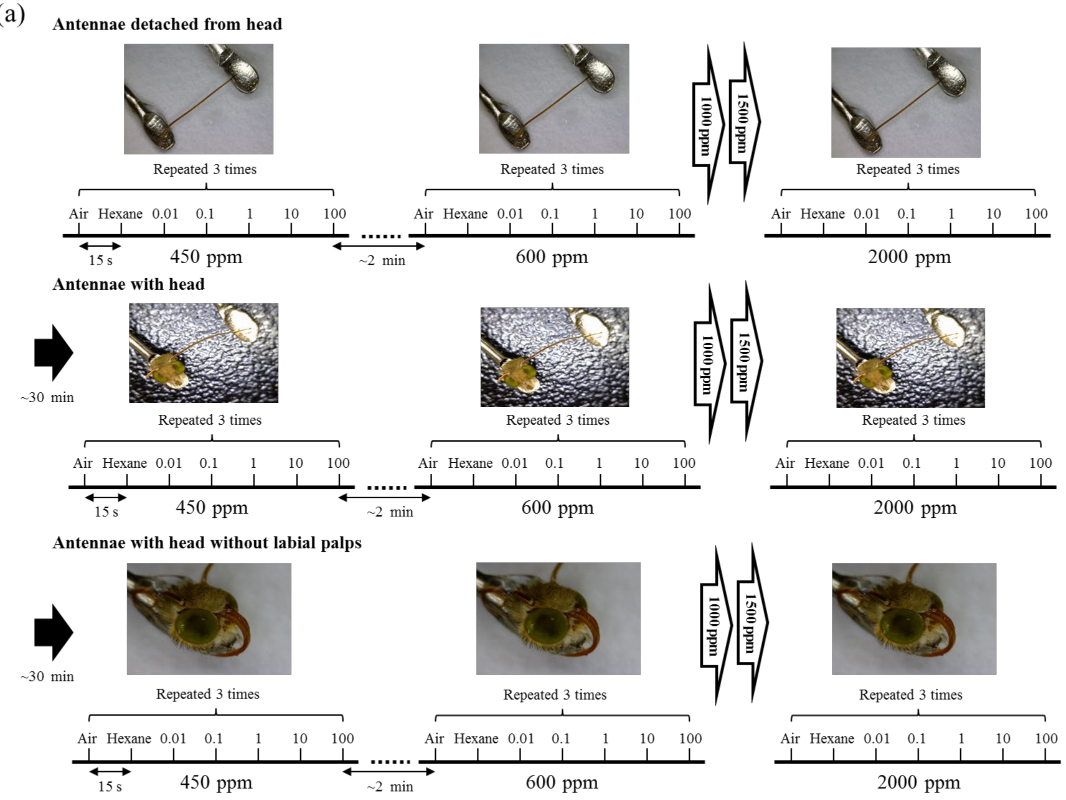
3. EAG test2 : for the male in increasing CO_2 level

H1 : Is there any change in male's antennal response as CO_2 levels increased?

H2 : Is there any change in the response between normal and labial palps removed male?

- Odor source: Control(Air, hexane), Treatment (0.01, 0.01, 0.1, 1, and 100 ug of Z11-16 Al)
- Samples : males are reared at 400 ppm of CO_2 level.
- EAG test: opposite antenna, head (antenna +head), labial palps removed head
- CO_2 treatment : 450 → 600 → 1000 → 1500 → 2000 ppm

(a)



(B)

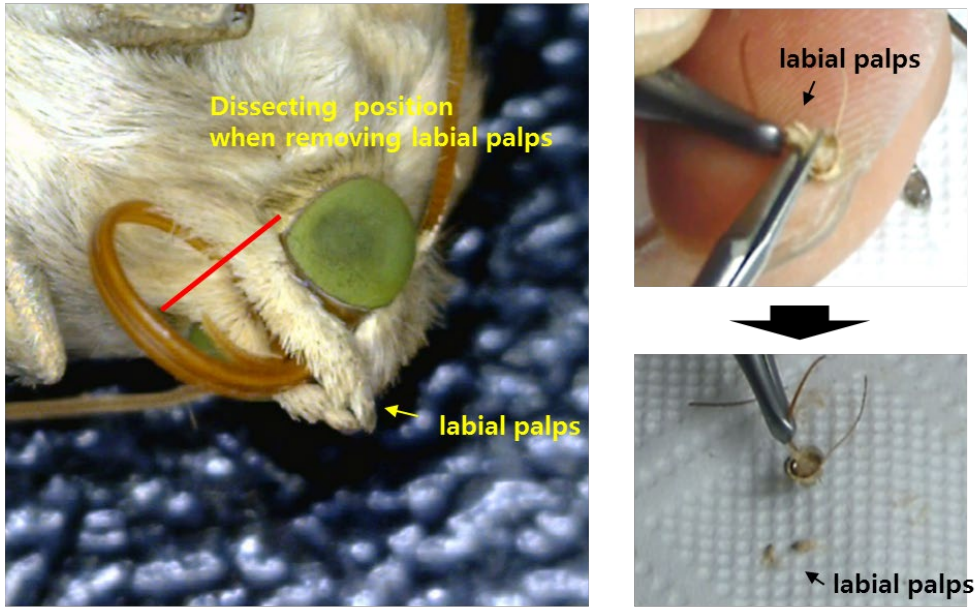


Figure S2. EAG experiment on male antenna, head, and head with the labial palps removed at changing CO_2 levels. (A) Experimental procedure for increasing CO_2 levels (450–2,000 ppm) and (B) Removal of the labial palps from the head.

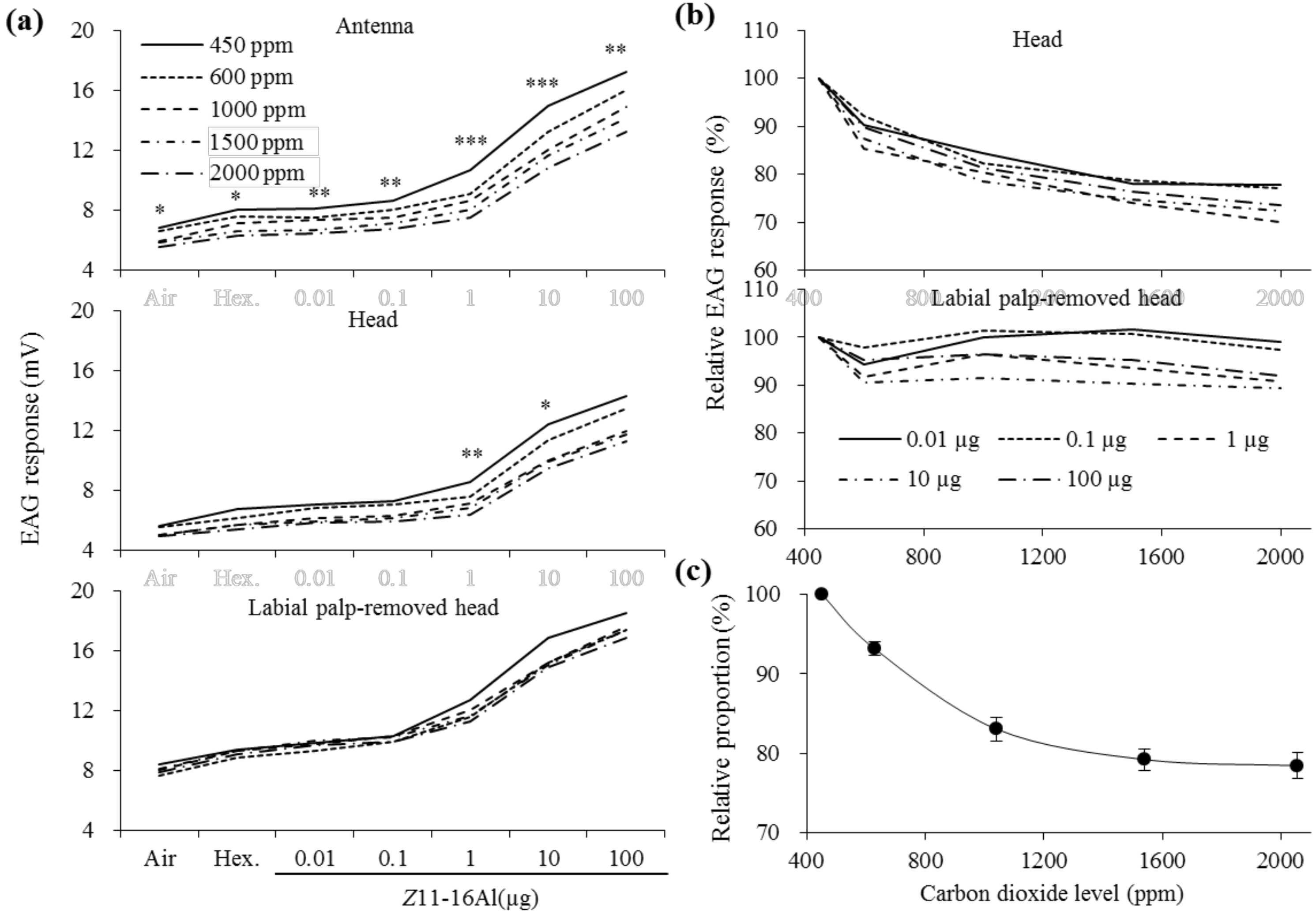
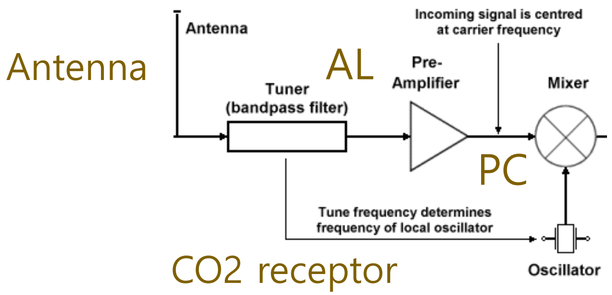


Figure 3. EAG response of *H. armigera* males (age 3 d) in increasing CO_2 levels to different concentrations of a synthetic sex pheromone component, *Z*11-16Al. Data are presented as means ± SE. (**A**) EAG test conducted with opposite antennae, heads, and heads with labial palps removed (n = 9; *: P < 0.05, **: P < 0.01, ***: P < 0.001). (**B**) Relative EAG response of whole heads and those with the labial palps removed, normalised to the response at control CO_2 level. **C**: Relative proportions of whole head responses to those of heads with the labial palps removed (df = 2, 220, F = 24.48, P < 0.0001).



- **EAGs of antenna was dependent on CO2 level while EAGs of labial palps removed head didn't**
- **EAGs of head showed a stable response under changing CO_2 levels rather than EAGs of antenna**

=> CO_2 receptor in labial palps may have a role like sieving/tuning signal from antenna

4. Sex pheromone production in Female

H3 : Is there any change in female's sex pheromone production?

- **Samples : females are reared at 400, 600, and 1000 ppm of CO_2 level and labial palp-removed female prepared just before scotophase.**
- **Pheromone extraction : 15 females at each time after scotophase (0, 2, 4, 6, 8, 10 h), Quantitification analysis : GC/MS**

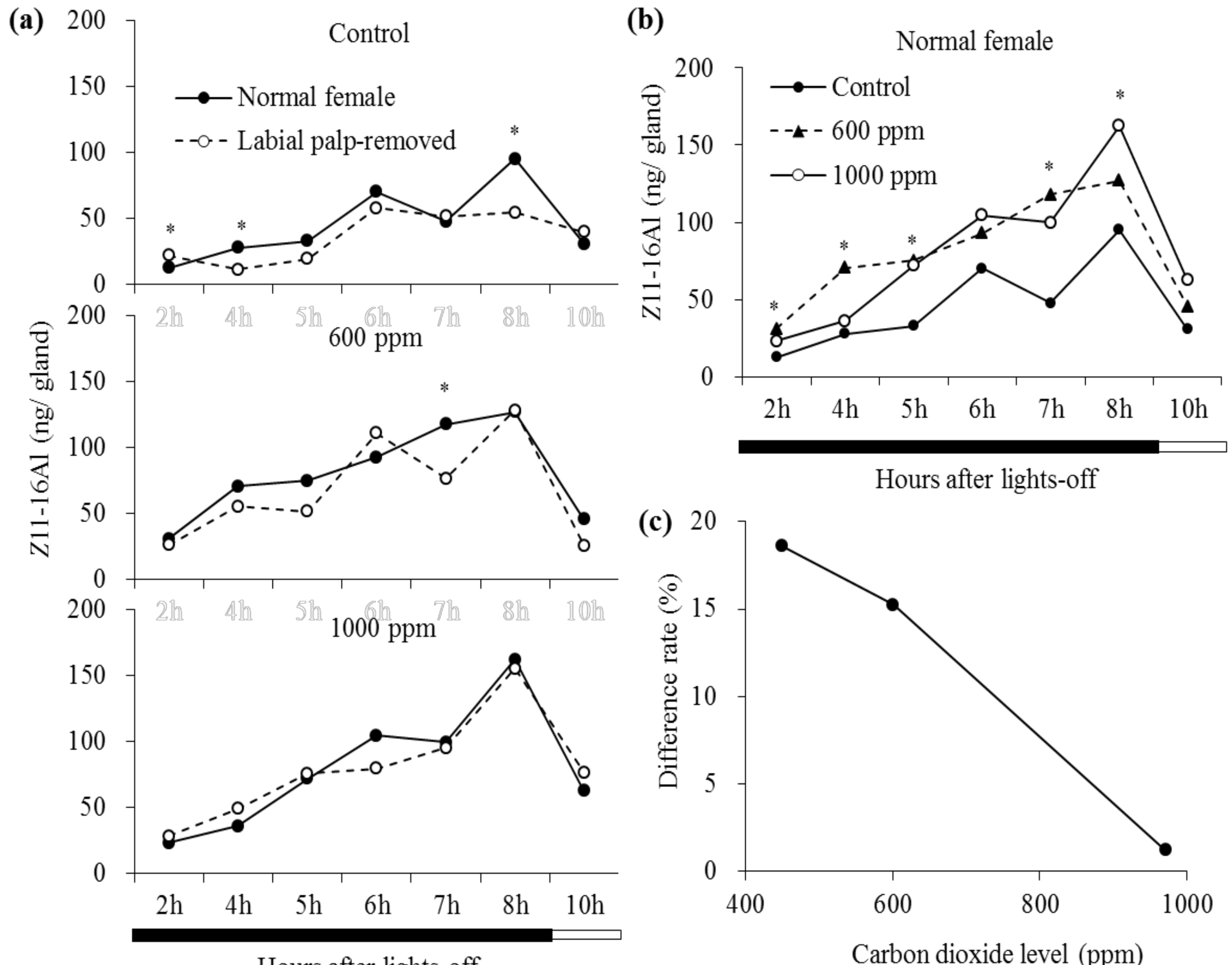


Figure 4. Sex pheromone production in *H. armigera* females under different CO_2 levels. Data are presented as means ± SE (■: scotophase, □: photophase) (*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$). (**A**) Comparison of the average amount of major sex pheromone component *Z*11-16Al produced per female reared at different CO_2 levels at intervals after lights-off between normal females and those with the labial palps removed (normal female: n = 103, control; labial palps removed: n = 104, 1,000 ppm; others: n = 105). (**B**) Changes in *Z*11-16Al production in normal females under different CO_2 levels. (**C**) Changes in the difference in total sex pheromone production between normal females and those with the labial palps removed at different CO_2 levels.

- **Pheromone production is affected by CO_2 level.**
- **CO_2 receptor seems to be involved in storage and emission process of PBAN.**

5. Mating

H 1,2,3 : Is there any change in mating?

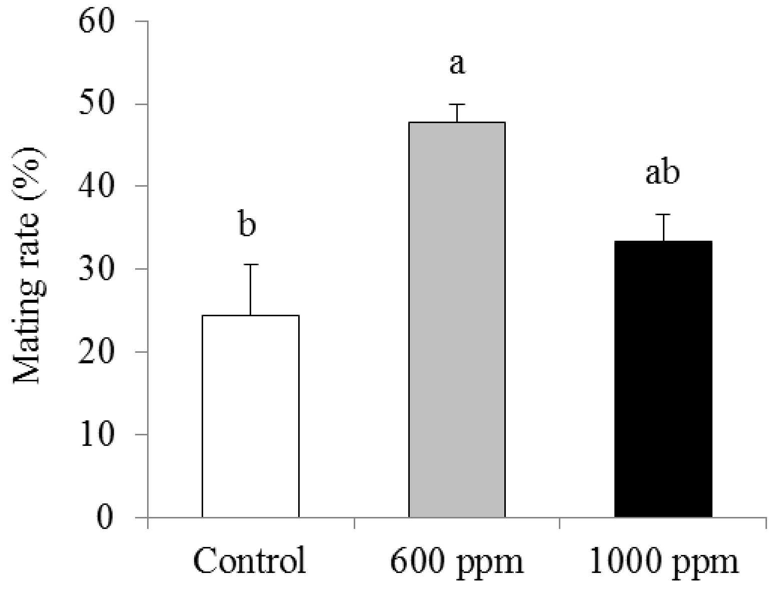
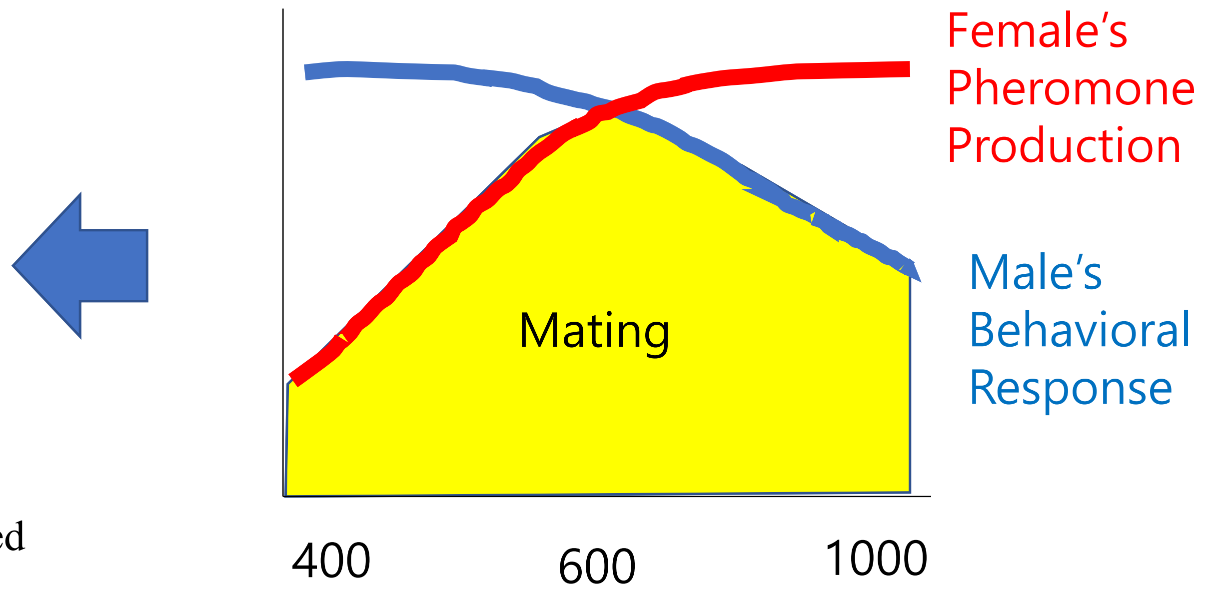
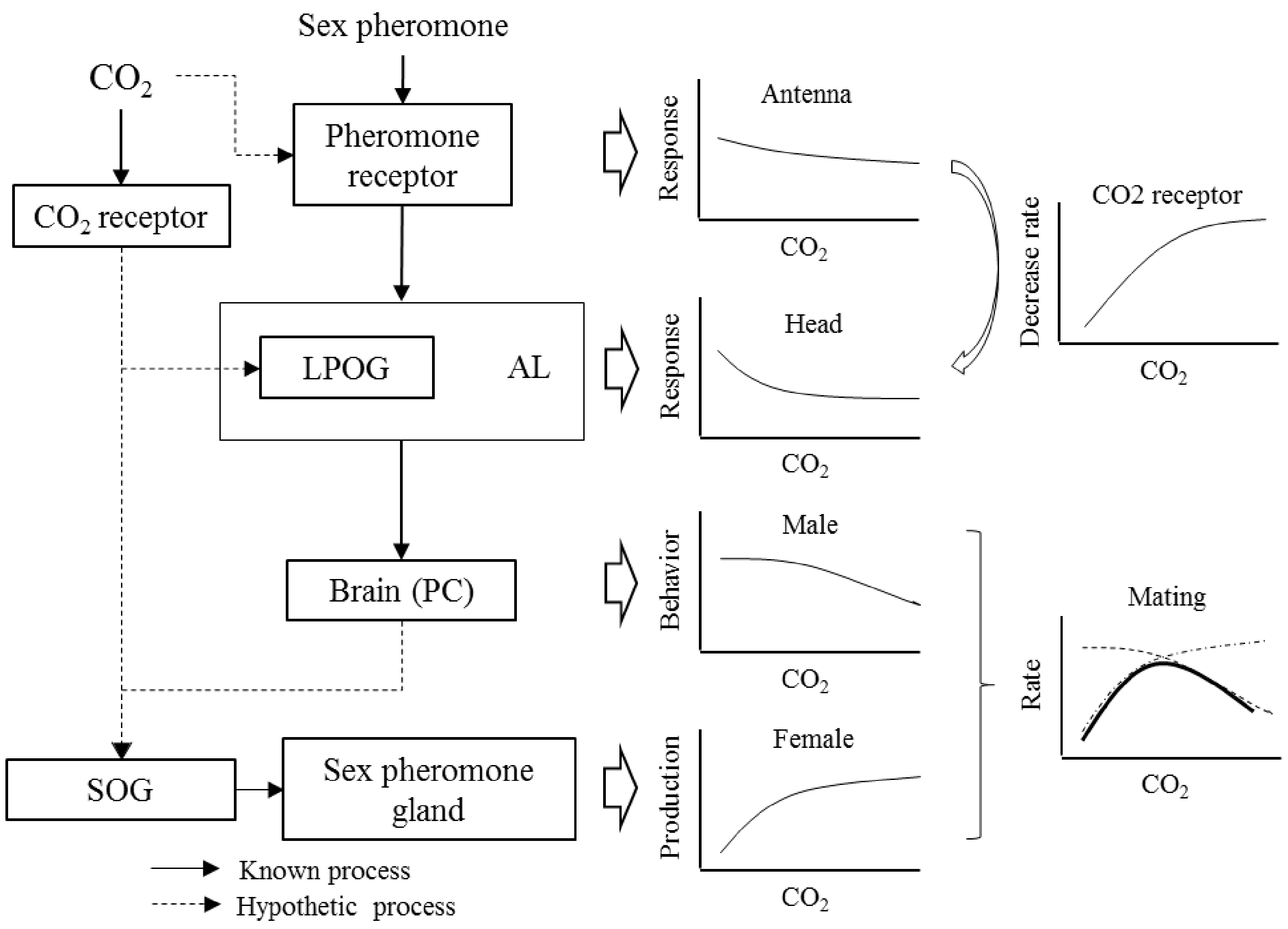


Figure 5. Mating rates of *H. armigera* adult pairs reared under different CO_2 levels. Data are presented as means ± SE (n = 90; df = 2, 26, F = 7.66, P = 0.0233).



※ Male and female adults are not aggregated together in nature like this mating experiment. Therefore, the reduction in detecting ability of male to sex pheromone would be an big handicap for male to hear the female's luring voice.

IV. Highlights



Ambient CO_2 concentration directly affects antenna receptors and their signal intensity decreases as the CO_2 level increases.

Male perception of the sex pheromone decreases at high CO_2, whereas females produce more sex pheromone with increased CO_2.

Finally, mating peaks at 600 ppm and decreases thereafter. CO_2 receptors located in the head have a role in mediating pheromone perception in males and production in females under various CO_2 environments

Thank you

In coming future

When CO_2 level increases

As much as the dinosaur had lived,

Lepidopteran would be a first insect to be extinct

No descendant know how beautiful butterfly was ……