

Plant stress and insect behavior:  
Cottonwood, ozone and the feeding  
and oviposition preference of a  
beetle

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# The reason I selected

- 卒論の時、ずいぶん助けていただいた論文
- This paper help me to write graduation thesis.

# Introduction

- 植物ストレスと虫の付きやすさには重要な関係がある
  - There is considerable interest in the relationships between plant stress and susceptibility to insects.
- 乾燥や栄養、大気汚染のストレスを受けた植物につく虫の摂食・産卵様式は変化する可能性
  - Insects on plants exposed to drought, nutrient or air pollution stress, might show changes in feeding or oviposition behavior.
- その変化は虫の分布や虫害を変化させる
  - The changes leads to differences in the distribution of the insect and the damage it causes.

# Previous study

- ハムシは弱光下で生育したヤナギの葉を好んだ
- A chrysomelid beetle preferred willow leaves grown in low light with optimal nutrients.
- インゲンテントウは  
二酸化硫黄やオゾン暴露を受けたマメ科の植物を好んだ
- The Mexican bean beetle preferred bean plants exposed to sulfur dioxide or ozone.
- マイマイガはオゾン暴露を受けたミズナラ苗に多様な反応を示した
- The gypsy moth showed a variable preference response to ozone-exposed oak seedlings.
- 産卵については蝶の仲間1種類で異なる養水分環境で育った  
アブラナ科の植物に対し、好みが変化した
- Oviposition behavior has been examined in one species of butterfly which showed varying preferences for Brassica plants grown under different water and nutrient regimes.

- 非生物ストレスを受けた植物への虫の摂食と産卵の嗜好を同時に調査した研究はない
- We are not aware of any studies that have simultaneously examined feeding and oviposition preferences for any insect on abiotically stressed plants.

# Purpose

- オゾン暴露したヤナギクローン2種に対するヤナギルリハムシの摂食と産卵の嗜好性を調査
- We examined the feeding and oviposition preference of the imported willow leaf beetle for 2 clones of cottonwood that had been exposed to a single acute dose of ozone or charcoal-filtered air.

# Materials and Methods

- アメリカクロヤマナラシ (*Populus deltoides*)
- 17～25葉期
- The 17-25 leaf stage
  
- さび病耐性クローンST66
- さび病非耐性クローンST109
- Resistant ST66 and susceptible ST 109 to leaf rust
  
- 温室土壌
- Greenhouse soil

# Ozone fumigation

- Open-top chamber
- July and August 1984
  
- Closed-top chamber
- July and August 1985 and 1986
  
- 8:00 ~ 13:00
- 0.20ppm
  
- Control (Charcoal-filtered air)



# Insect collection

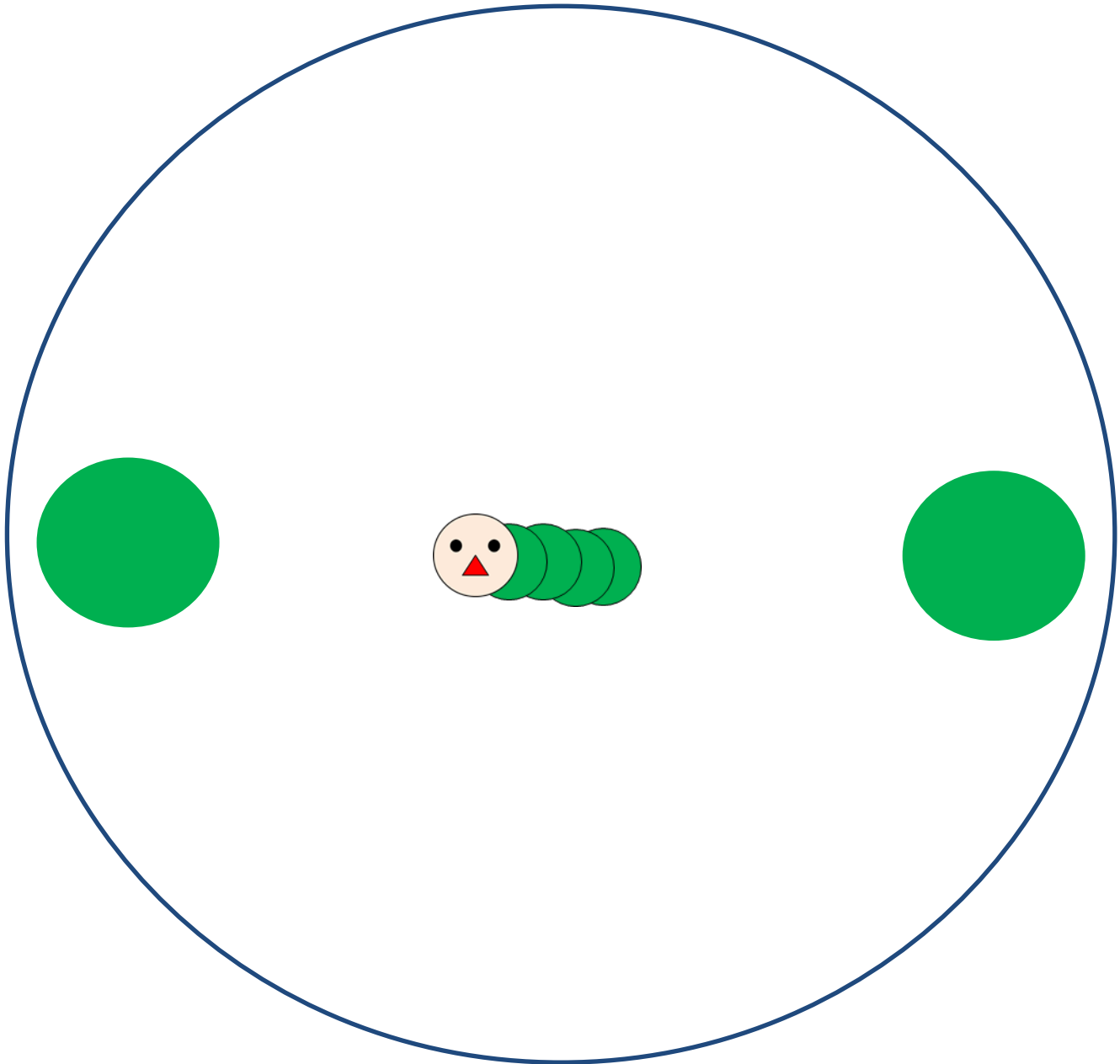
- ブラックウィロー(ヤナギ科)に付いてる野生のヤナギハムシを採集
  - *P. versicolora* were collected from single wild population on *Salix nigra*.
- 二齡幼虫(一回目の脱皮後の幼虫)を実験内で採集
  - Second instar larvae were collected on the day of the experiment

# Previous experiment

- 成虫と幼虫ともに第5葉の葉を最も好んだ
- Previous experiments demonstrated that on both clones, LPI-5, the leaf approaching full expansion, was most highly preferred for feeding by beetle adults and larvae.
  
- 分析評価には着葉していた  
葉のリーフディスクと全葉を使用
- Assays using leaf discs and whole leaves attached to the plant.

# Dual-choice leaf disc feeding preference assay

- リーフディスク: 17 mm
- Discs 17mm in diameter
- O<sub>3</sub>葉とコントロール葉のディスクを直径60 mmと100 mm 深さ15 mmのシャーレに対にして配置
- Treated and control disc were placed abaxial side up at opposite sides of a 60 mm (larvae) or 100 mm (adult) dia × 15 mm deep Petri dish, containing a filter paper moistened with deionized water.
- 幼虫と成虫それぞれ1匹ずつシャーレの中央に配置
- A single adult and larva was placed in the center of each replicate Petri dish.
- 環境制御室にて実施
- Plates were placed in a controlled environment chamber.



- 葉面積の消費割合は毎1時間計測を4時間  
それから12時間ごとに計測を7日間  
もしくはディスクが完全に消費されるまで
- Percent leaf area consumed from each disc was visually estimated every hour for 4 hours, then every 12 hours for 7 days, or until 230 mm<sup>2</sup> was consumed in the arena.
- 先に50 mm<sup>2</sup>消費された方がより好まれたもの  
とした
- A disc was considered preferred for feeding if it was the first disc to have 50 mm<sup>2</sup> consumed.

## Dual-choice leaf disc oviposition preference assay

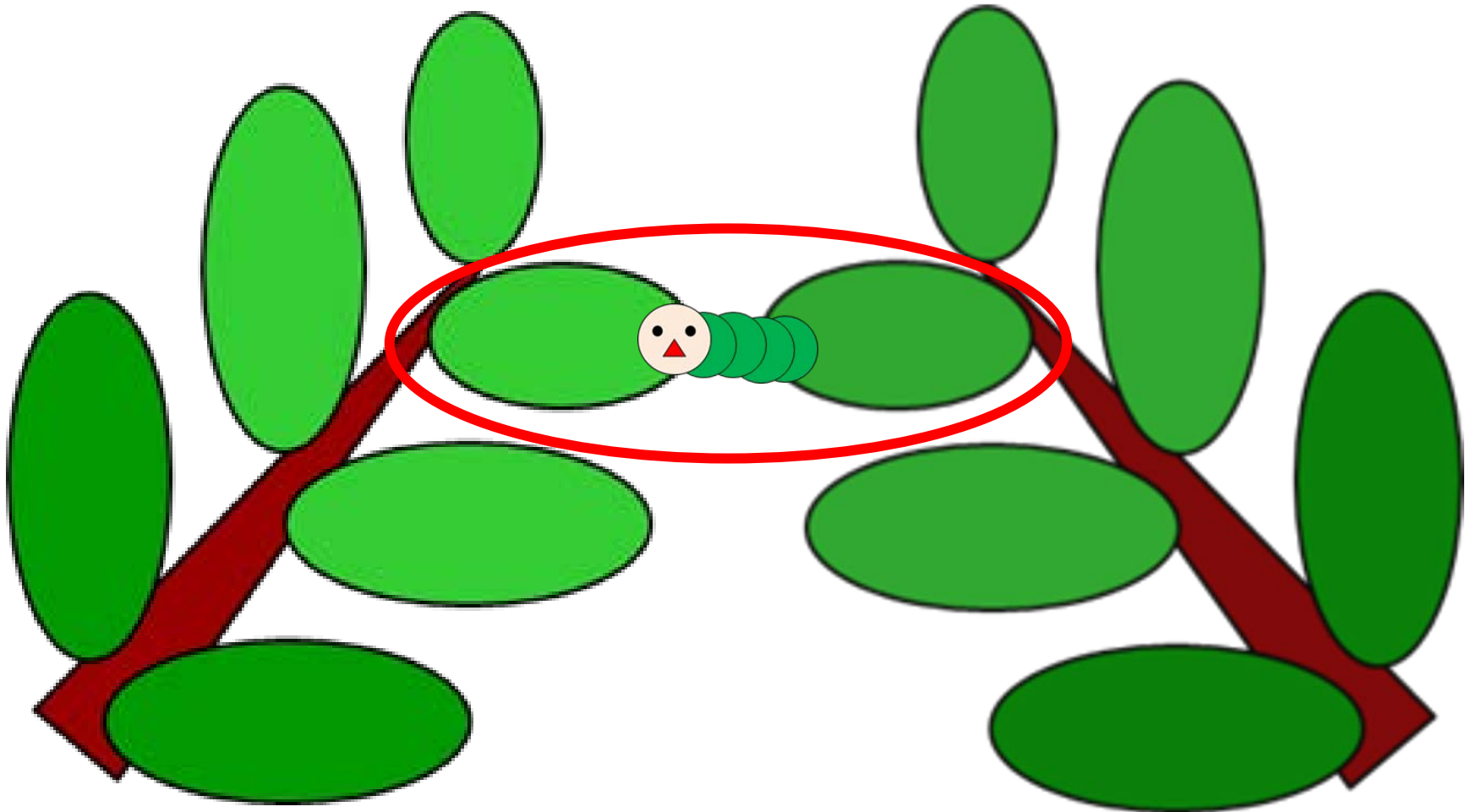
- Same design as the feeding assay

# Dual-choice attached leaf assay

- 2処理のヤナギの第5葉の周りを一緒に寒冷紗で包んだ
  - A cheesecloth bag was stapled around LPI-5 of each plant pair so as to enclose both leaves.
- 24時間ごとに葉面積の消費割合を計測
  - Leaf area consumed of each leaf on the 15 replicates was estimated every 24 h.
- 先に100 mm<sup>2</sup>消費された方がより好まれたものとした
  - A leaf was considered preferred for feeding if it was the first to have 100 mm<sup>2</sup> consumed.

$O_3$

control





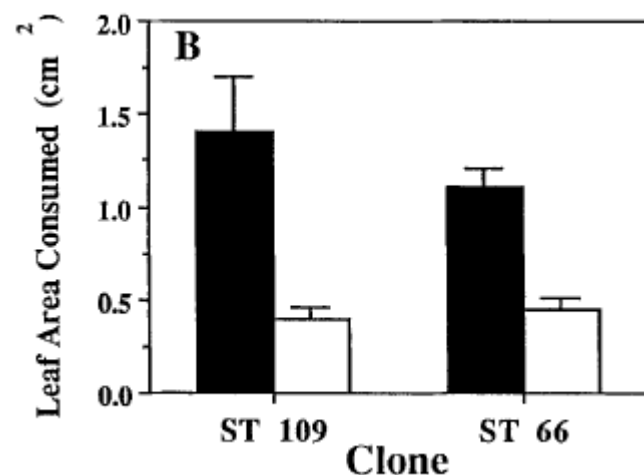
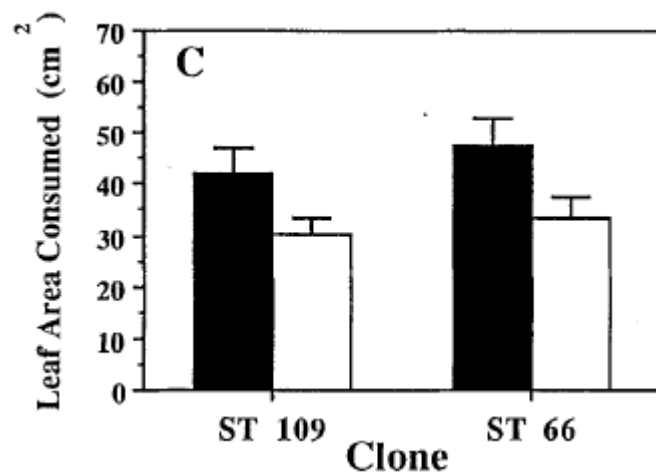
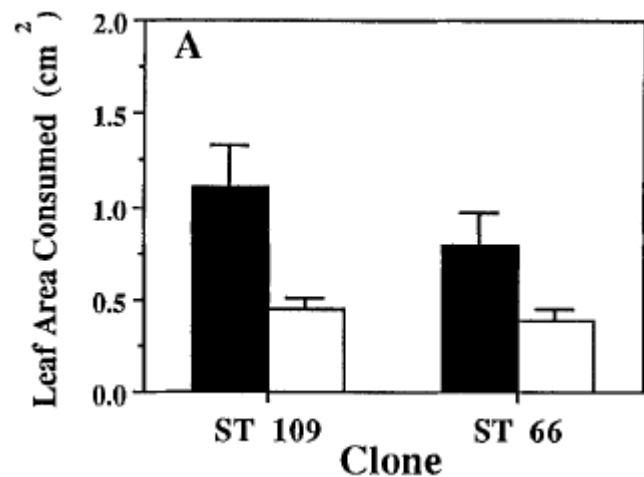
## Multiple-choice whole plant feeding and oviposition preference assay

- 浄化空気の温室にて実施
  - Carried out in a CFA greenhouse and used 15 plants treatment of each clone in 1986.
- 2処理のヤナギ3個体ずつ木製のケージに設置し、雄雌成虫15匹ずつ中央に配置
  - Treated and control plants were randomly assigned to one of five replicate aluminum-screened wooden cages, with 3 treated and 3 control plants/cage randomly distributed with overlapping leaves.
  - Fifteen male and 15 female adult beetles were placed in 2 Petri dish halves in the center of the cage.
- 24時間ごとに葉面積の消費割合と産卵数を測定
  - Percentage leaf area consumed was estimated for each leaf, and egg mass distribution was recorded every 24 h.
- 14日後にすべての葉を回収、消費割合を測定
  - After 14 days each leaf was removed and the amount of damage visually estimated and confirmed, using the same procedure as with the attached leaf assay.

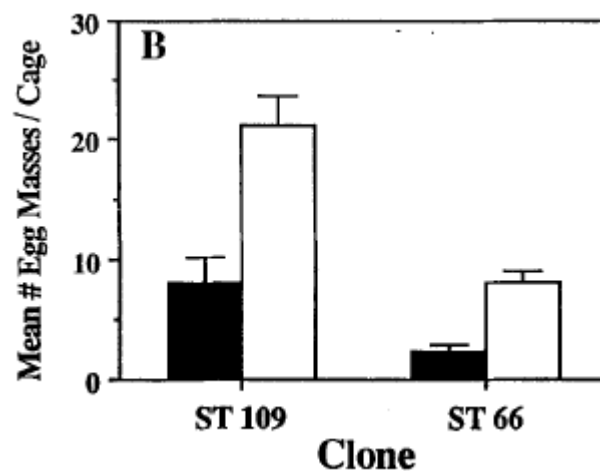
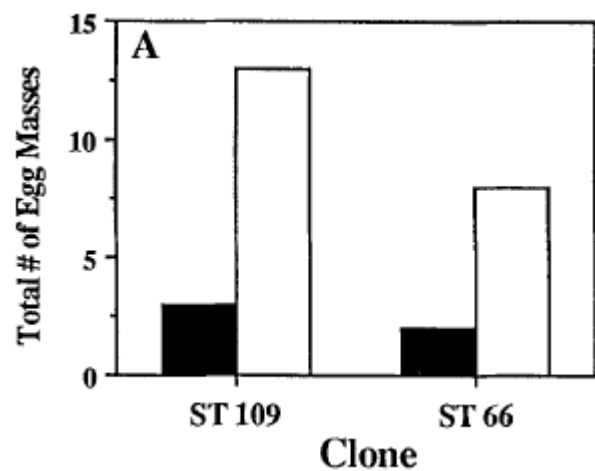
# Result

**Table 1.** Feeding preference of *P. versicolora* adults (A) and second-instar larvae (L) for cottonwood leaves of two clones (ST109, ST66) treated with ozone (O<sub>3</sub>) or charcoal-filtered air (CFA) in dual-choice leaf disc (disc) or attached leaf (leaf) assays

Assay	Clone	Insect Stage	Year	Number of Replicates	Beetle Preference Ratio (O <sub>3</sub> :CFA)	Sign Test Significance Level
Disc	ST109	L	1986	20	6 :1	0.05
Disc	ST109	L	1986	24	6 :1	0.05
Disc	ST109	L	1984	24	6 :1	0.05
Disc	ST109	A	1986	20	2.8:1	0.1
Disc	ST109	A	1985	60	5.5:1	0.01
Disc	ST109	A	1984	36	6 :1	0.05
Disc	ST66	L	1986	20	6 :1	0.05
Disc	ST66	L	1986	20	5.7:1	0.05
Disc	ST66	A	1985	60	4.3:1	0.05
Disc	ST66	A	1984	20	5.5:1	0.05
Leaf	ST109	L	1986	15	6.5:1	0.05
Leaf	ST66	L	1986	15	6.5:1	0.05



**Fig. 1 A–C.** Mean leaf area consumed ( $\pm 1$  S.E.) by adult *P. versicolora* in choice tests with cottonwood of two clones (ST109, ST66), treated with ozone (■) or charcoal-filtered air (□) in different trials: **A** Consumption after 7 days in a dual-choice disc assay, 1984. **B** Consumption after 7 days in a dual-choice disc assay, 1985. **C** Consumption after 14 days in a multiple-choice whole plant assay, 1986



**Fig. 2 A, B.** Oviposition by *P. versicolora* females on cottonwood of 2 clones (ST109, ST66) treated with ozone (■) or charcoal-filtered air (□) in different trials: **A** Total number of masses in all dual-choice leaf disc assays, 1984, 1985, and 1986. **B** Mean number of masses per cage ( $n = 5$  replicate cages,  $\pm 1$  S.E.) in multiple-choice whole plant assay, 1986

# Discussion

- O<sub>3</sub>感受性の高い樹木はO<sub>3</sub>暴露を受けるにつれ大きな虫害に遭う可能性
- First, trees showing the greatest susceptibility to ozone stress could also experience the greatest amount of beetle damage immediately following the ozone exposure.
- O<sub>3</sub>感受性の高い樹木は他の樹木に産卵されることで次の世代の虫害が減少する可能性
- However, the subsequent generation(s) of beetles that would have normally been oviposited on these plants would be oviposited elsewhere and the ozone-susceptible plants should then be exposed to less subsequent beetle damage.

- O<sub>3</sub>感受性の高い樹木の傍に生育する樹木は初めは虫害が減少する可能性
- O<sub>3</sub>感受性の高い樹木への産卵を避けた成虫により通常よりも産卵数が増加
- 次の世代の虫により激しい虫害に遭う可能性
- Second, those trees that are growing near susceptible plants but are not themselves susceptible to ozone stress might gain an initial benefit from a reduction in beetle consumption as the adults moved to stressed plants, but would then, ironically, receive a subsequently greater oviposition load following the acute ozone episode as beetles moved away from stressed plants to oviposit on them instead.
- This greater egg load might subsequently lead to greater damage to ozone-resistant plants, an unexpected consequence of being resistant to stress.

- O<sub>3</sub>によってヤナギにおける虫や菌との関係に変化が生じる可能性
- それら虫や菌からの被害も変化する可能性
- Third, it is also possible that there might be alterations in the structure of the insect and pathogen community on cottonwood, and the damage to the plant that these other organisms cause, following an acute ozone episode.

Thank you!

