IAEA 2023
2023.9.19~22, IAEA Headquarters, Vienna, Austria.

Plant Pathogens Control Using Air Atmospheric Pressure Plasmas

Toshiro Kaneko, Shota Sasaki, Keisuke Takashima,

Graduate School of Engineering, Tohoku University, Japan

Green Food System Strategy (2021. 5. 12)

令和3年5月 農林水産省

Ministry of Agriculture, Forestry and Fisheries of Japan

現状と今後の課題

○生産者の減少・高齢化、
地
○温
○コロナを契機としたサプライチェーン混乱、内食拡大
○SDGsや環境への対応強化
○国際ルールメーキングへの参画

「Farm to Fork戦略」(20.5)
2030年までに化学農薬の使用及びリスクを50%減、有機農業を25%に拡大

「農業イノベーションアジェンダ」(20.2)
2050年までに農業生産量40%増加と環境フットプリント半減

農林水産業や地域の将来も見据えた持続可能な食料システムの構築が急務

持続可能な食料システムの構築に向け、「みどりの食料システム戦略」を策定し、中長期的な観点から、調達、生産、加工・流通、消費の各段階の取組とカーボンニュートラル等の

目指

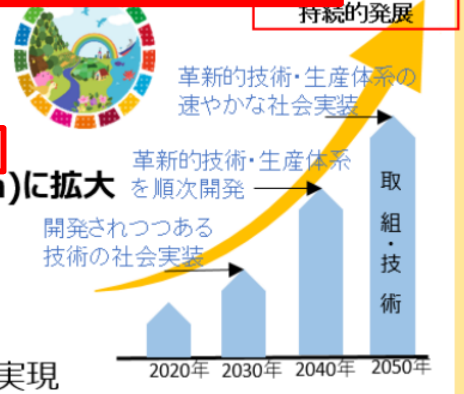
2050年までに目指す姿

Low risk pesticides

50% reduction in **chemical pesticide** use

30% reduction in **chemical fertilizer** use

- 農林水産業の**CO2ゼロエミッション化**の実
- 低リスク農薬への転換、総合的な病害虫管理…の確立・普及に加え、ネオニコチノイド系を含む従来の殺虫剤に代わる新規農薬等の開発により**化学農薬の使用量（リスク換算）を50%低減**
- **輸入原料や化石燃料を原料とした化学肥料の使用量を30%低減**
- 耕地面積に占める**有機農業の取組面積の割合を25%(100万ha)に拡大**
- 2030年までに**食品製造業の労働生産性を…割向上**
- 2030年までに食品企業における…**に配慮**した
…**比率100%**を実現



2040年までに革新的な技術・生産体系を順次開発（技術開発目標）
2050年までに革新的な技術・生産体系の開発を踏まえ、
今後、「政策手法のグリーン化」を推進し、その社会実装を実現（社会実装目標）
※政策手法のグリーン化：2030年までに施策の支援対象を持続可能な食料・農林水産業を行う者に集中。
2040年までに技術開発の状況を踏まえつつ、補助事業についてカーボンニュートラルに対応することを目指す。
補助金拡充、環境負荷軽減メニューの充実とセットでクロスコンプライアンス要件を充実。
※ 革新的技術・生産体系の社会実装や、持続可能な取組を後押しする観点から、その時点において必要な規制を見直し。
地産地消型エネルギーシステムの構築に向けて必要な規制を見直し。

期待される効果

経済　持続的な産業基盤の構築
・輸入から国内生産への転換（肥料・飼料・原料調達）
・国産品の評価向上による輸出拡大
・新技術を活かした多様な働き方、生産者のすそ野の拡大

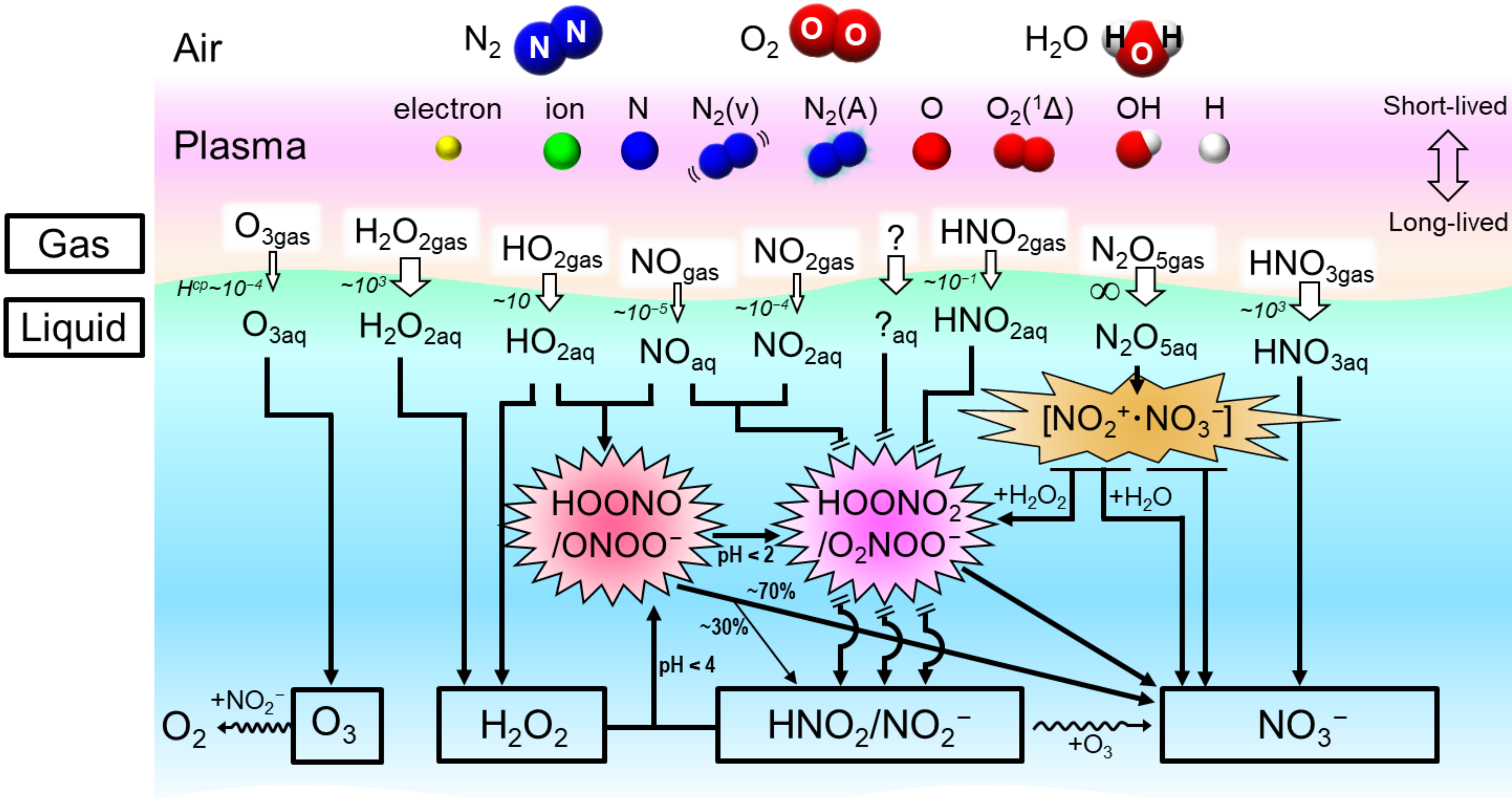
社会　国民の豊かな食生活 地域の雇用・所得増大
・生産者・消費者が連携した健康的な日本型食生活
・地域資源を活かした地域経済循環
・多様な人々が共生する地域社会

環境　将来にわたり安心して暮らせる地球環境の継承
・環境と調和した食料・農林水産業
・化石燃料からの切替によるカーボンニュートラルへの貢献
・化学農薬・化学肥料の抑制によるコスト低減

アジアモンスーン地域の持続的な食料システムのモデルとして打ち出し、国際ルールメーキングに参画（国連食料システムサミット（2021年9月）など）

Air APP Induced Liquid Phase Chemistry

Air Atmospheric Pressure Plasma (Air APP)

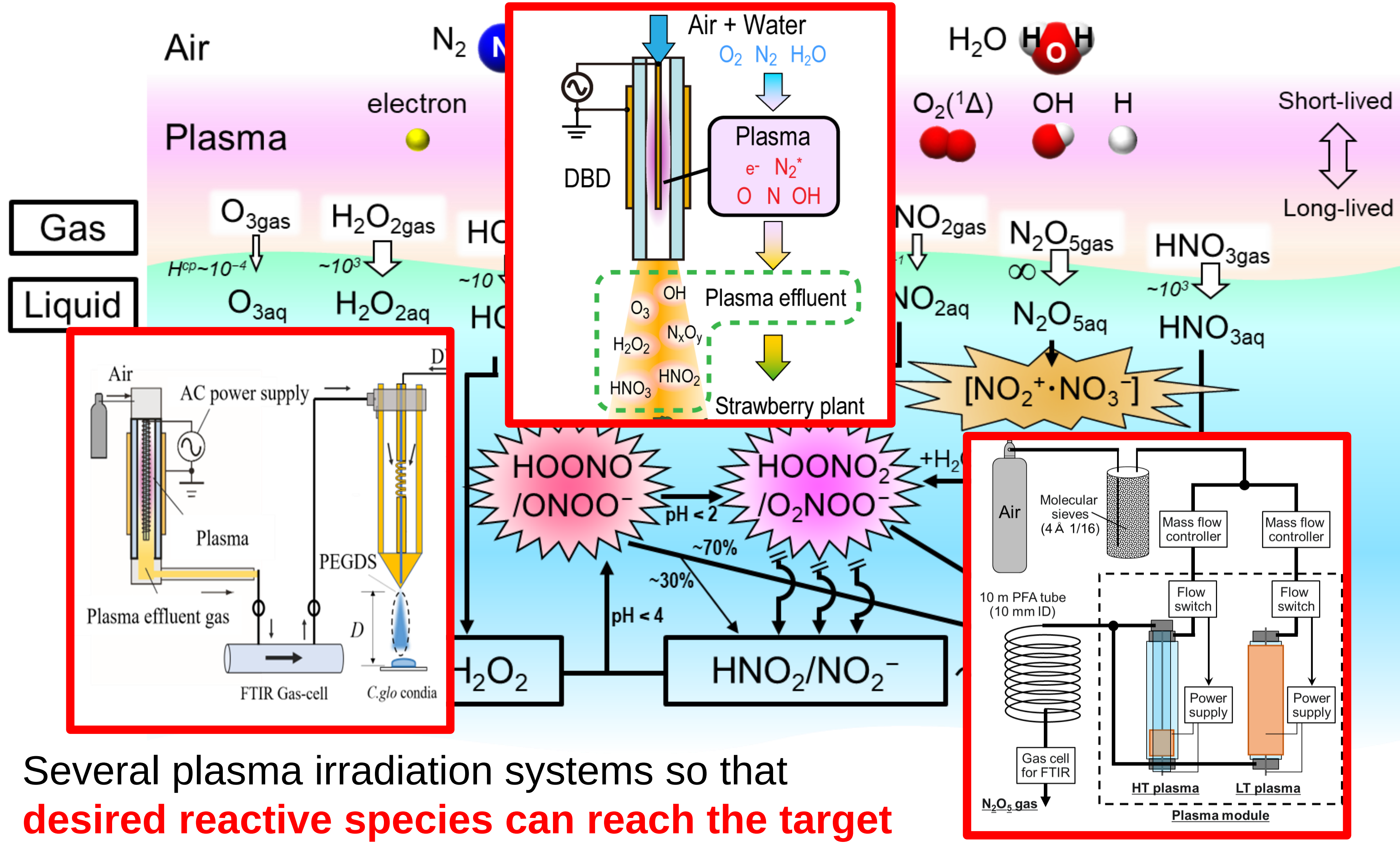


Several plasma irradiation systems so that

desired reactive species can reach the target

Air APP Induced Liquid Phase Chemistry

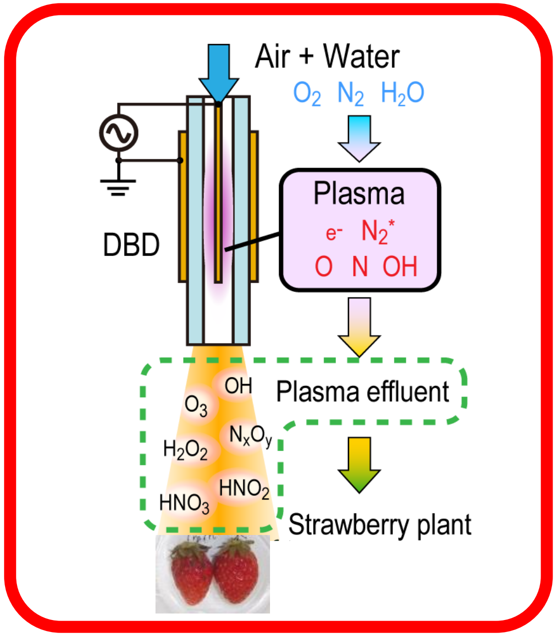
Air Atmospheric Pressure Plasma (Air APP)

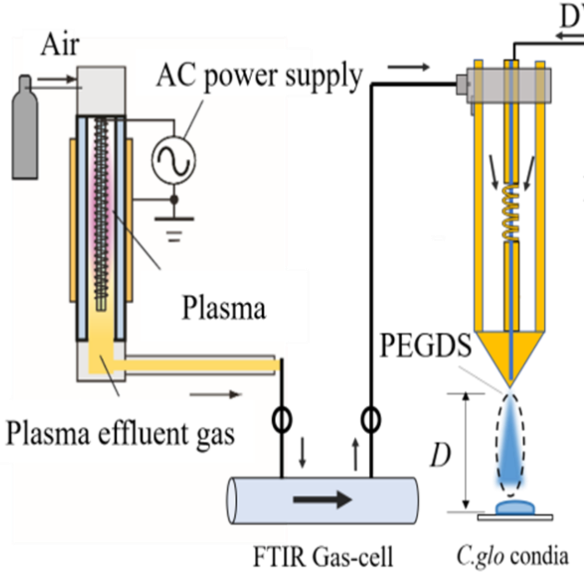


Several plasma irradiation systems so that **desired reactive species can reach the target**

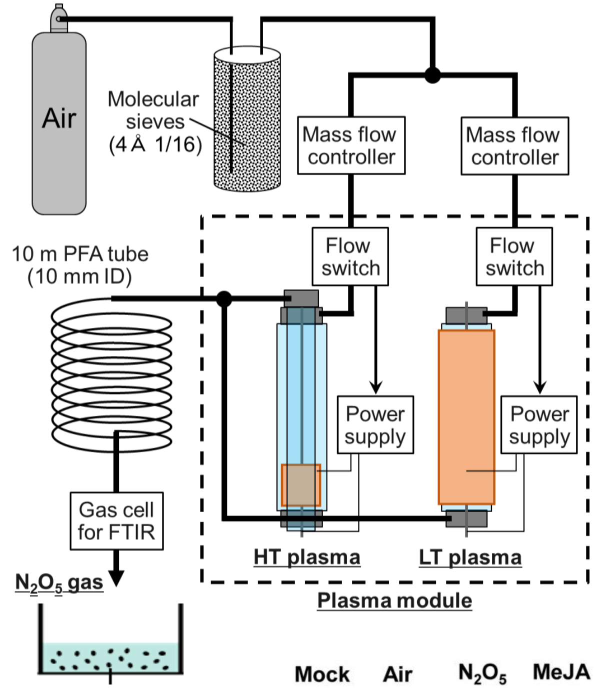
Air APP Irradiation System : Agriculture Applications

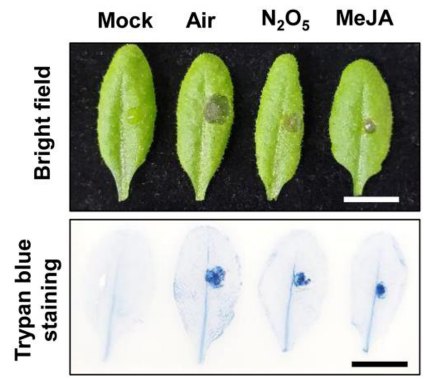
【sterilization/virus inactivation】 (during cultivation)





【plant immunity activation】 【nitrogen fertilization】

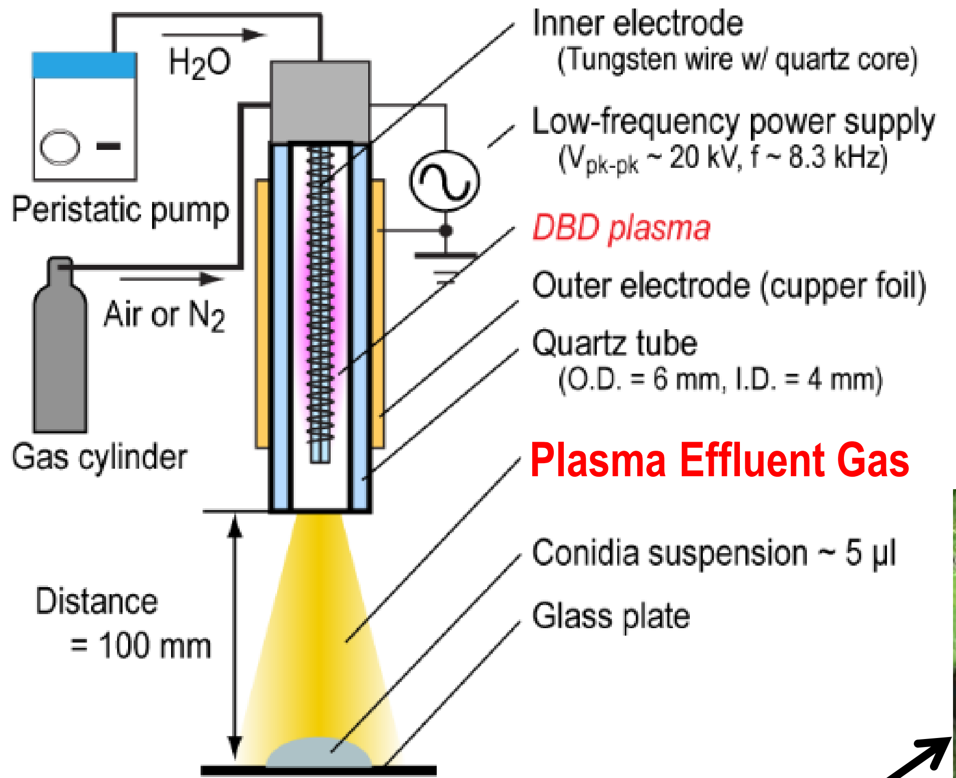




T. Kaneko, K. Ishikawa, et al., Jpn. J. Appl. Phys. **61**, SA0805 (2022)

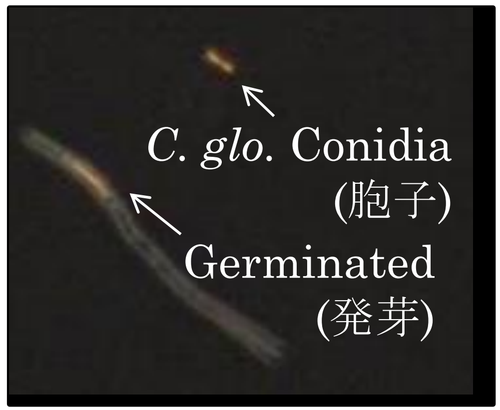
Air APP Effluent Spray System for Pathogen Control

Air Atmospheric Pressure Plasma (APP)



Experimental Condition

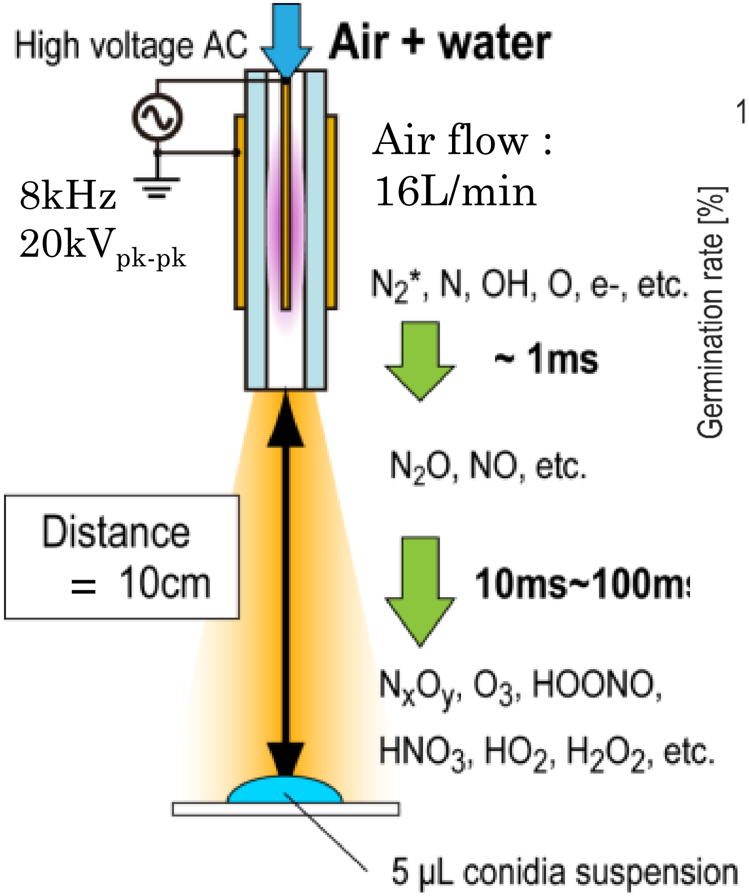
Gas	Air
Voltage ($V_{p\text{-}p}$)	20 kV
Frequency (f)	8.3 kHz
Irradiation Time (T)	0 ~ 120 sec
Irradiation Length (L)	100 mm
Air flow rate (F_{air})	4 ~ 16 L/min
Water flow rate (F_{H_2O})	0 ~ 150 µl/min

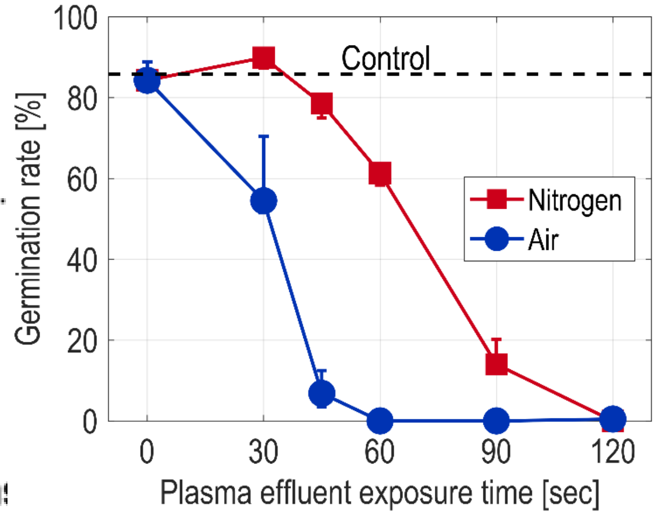


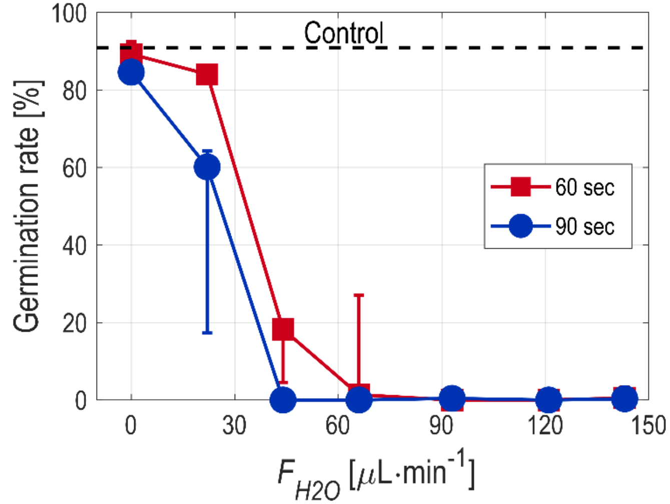
Strawberry pathogen:
Colletotrichum gloeosporioides (C. glo.)

$$\text{Germination rate} = \frac{\textit{Germinated conidia}}{\textit{Total conidia}}$$

Germination Suppression (Sterilization) by Air APP





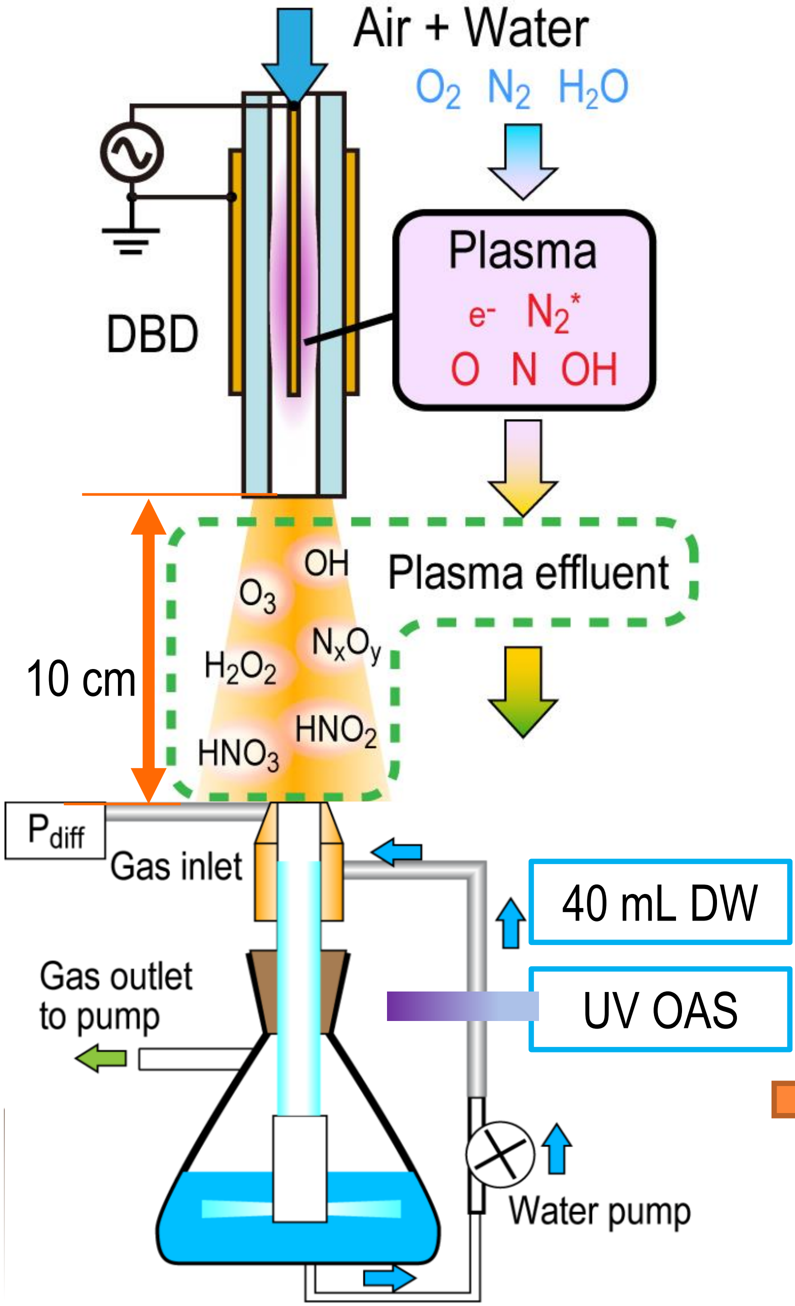


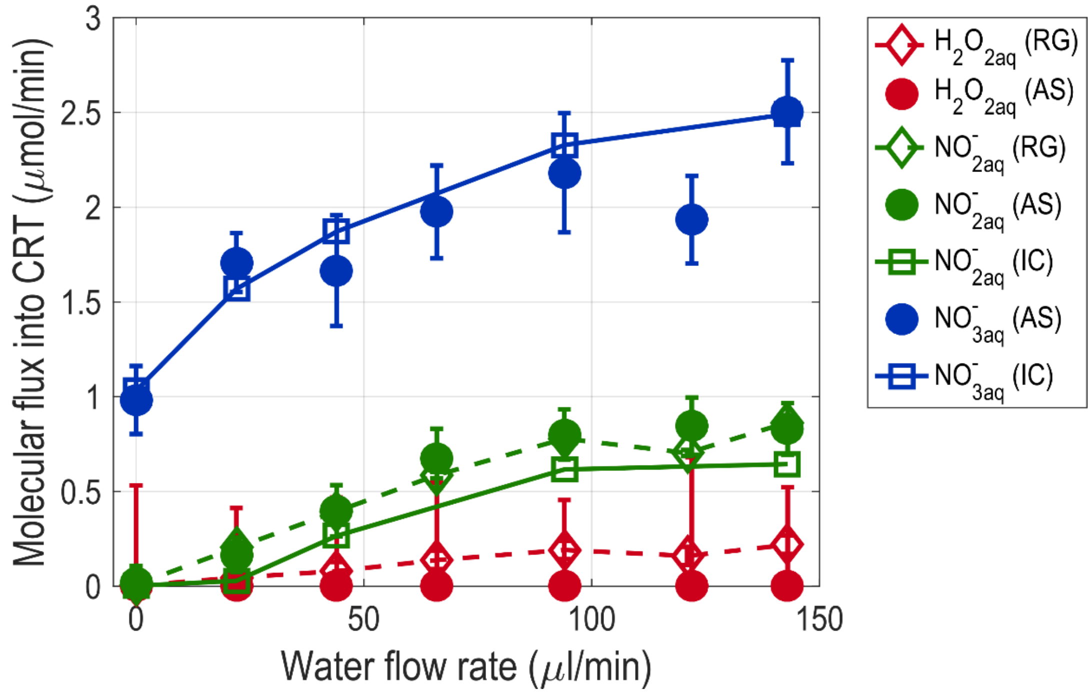
- Conidia suspension
  - 5uL conidia suspension with density of 2×10^6 conidia/ml in DW
  - 6~12 hours cultivation under 28 ˚C

- Germination rate gradually decreases with an increase in the plasma irradiation time
- Germination rate drastically decreases with an increase in the water flow rate

K. Shimada, K. Takashima, Y. Kimura, K. Nihei, H. Konishi, and T. Kaneko: Plasma Process. Polym., **17**, e1900004 (2020).

Key Reactive Species in Solution





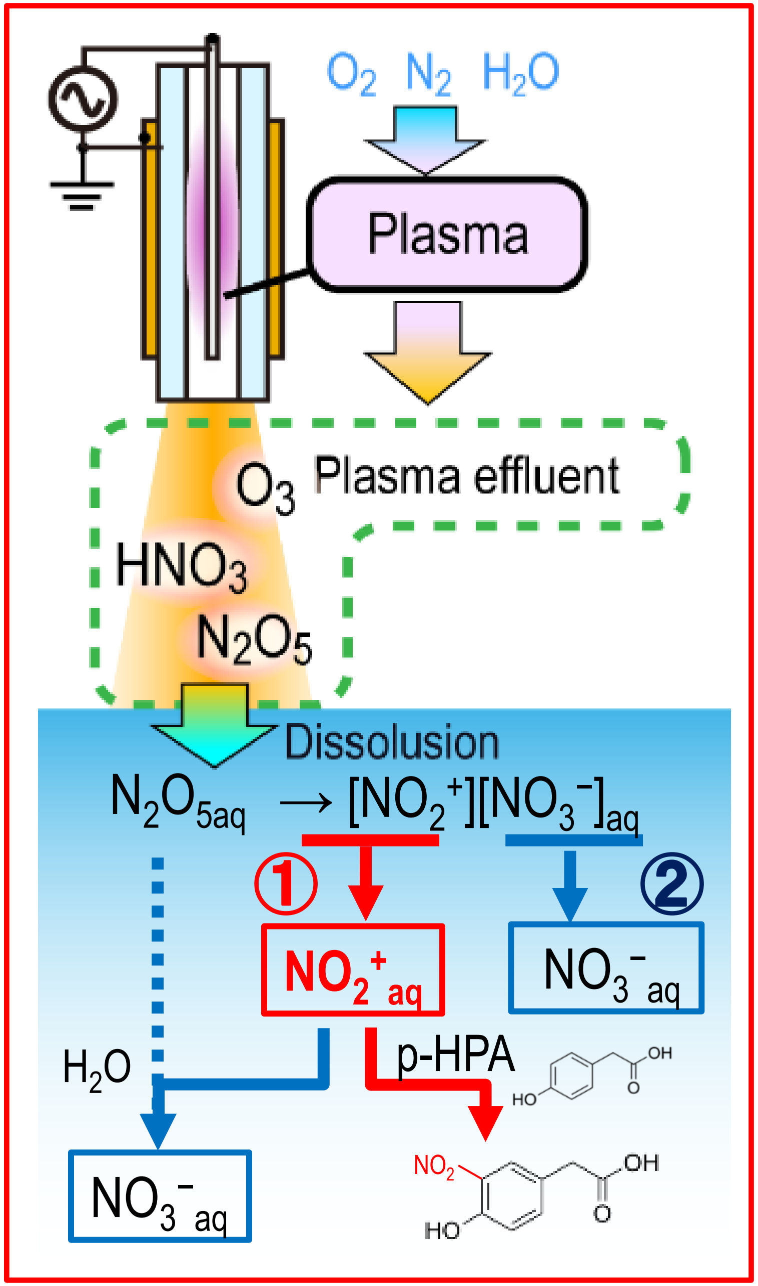
- H_2O in plasma acts as a hydrogen source and enhances dissolution of reactive species in liquid phase, related with pathogen germination suppression.

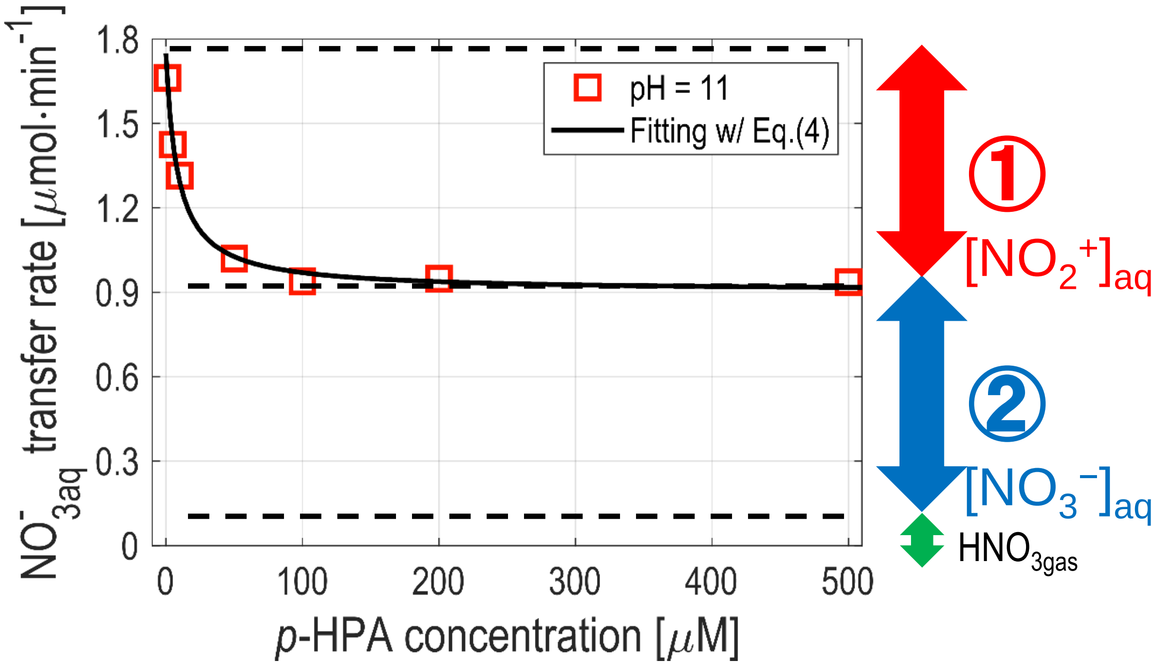
$$H_2O_{2aq} + HNO_{2\,aq} + H^+_{aq} \rightarrow \textbf{HOONO}_{\textbf{aq}} + H_2O_{aq} + H^+_{aq}$$

Y. Kimura, K. Takashima, S. Sasaki, and T. Kaneko: *J. Phys. D*, **52**, 064003 (2019)

P. Lukes et al, *Plasma Sources Sci. Technol.*, **23**, 015019 (2014)

$NO_2{}^+{}_{aq}$ Production from N_2O_{5aq}





- p-HPA is $NO_2{}^+{}_{aq}$ scavenger.
- Nearly 50% of $NO_3{}^-{}_{aq}$ generation is reduced by p-HPA

$NO_2{}^+$: Nitronium Ion

N_2O_5 : Dinitrogen Pentoxide

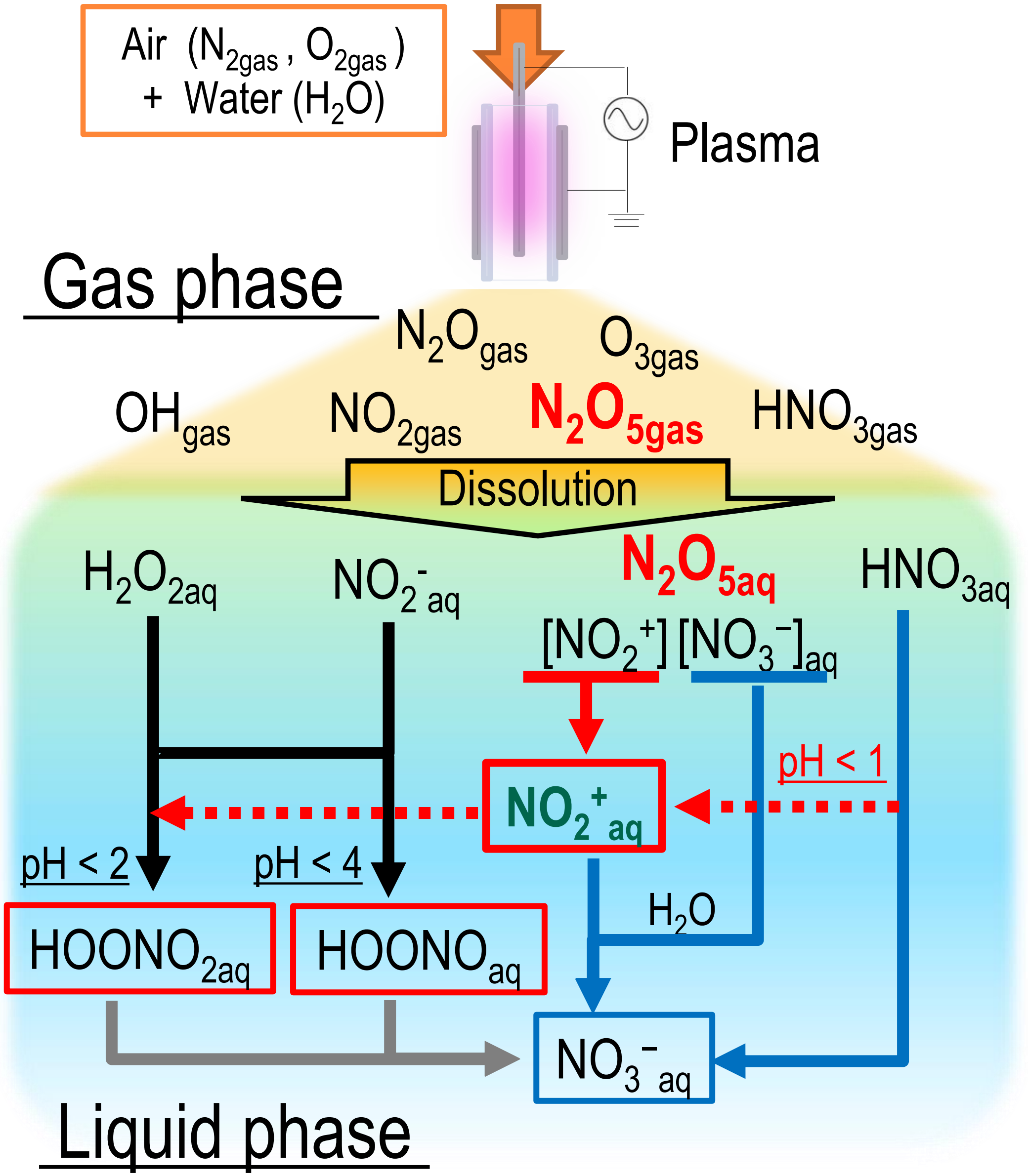
Reactive Oxygen/Nitrogen Species for Sterilization

Gas Phase

- N_2O_{5gas}, O_{3gas}, NO_{2gas}, etc…

Liquid Phase

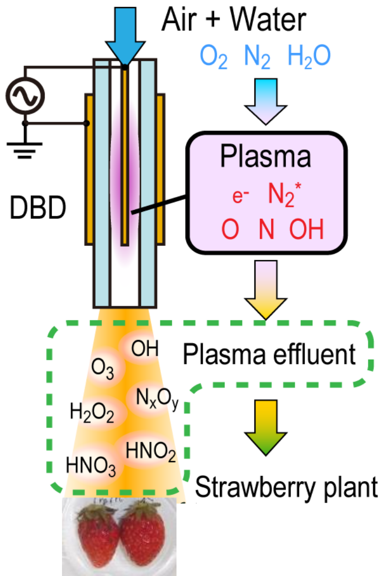
- $NO_2{}^+{}_{aq}$ production from N_2O_{5aq}
- $N_2O_{5aq} + H_2O_{2aq} \rightarrow$ $HOONO_{2aq} + H^+{}_{aq} + NO_3{}^-{}_{aq}$
- Highly reactive species: $NO_2{}^+{}_{aq}$ and $HOONO_{2aq}$

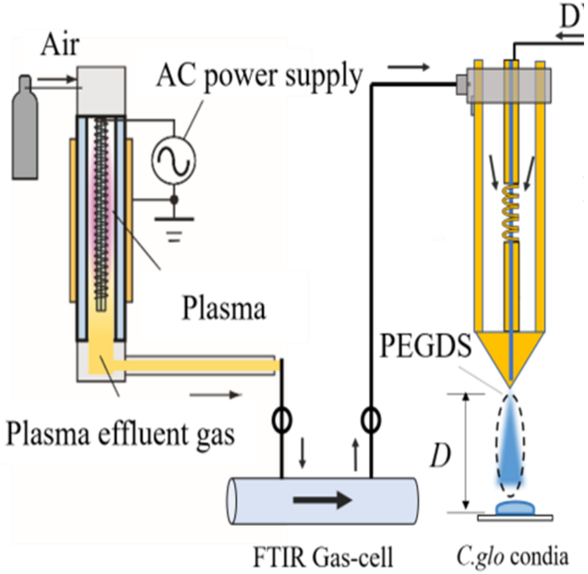


Y. Kimura, K. Takashima, S. Sasaki, and T. Kaneko: J. Phys. D: Appl .Phys., **52**, 064003 (2019)

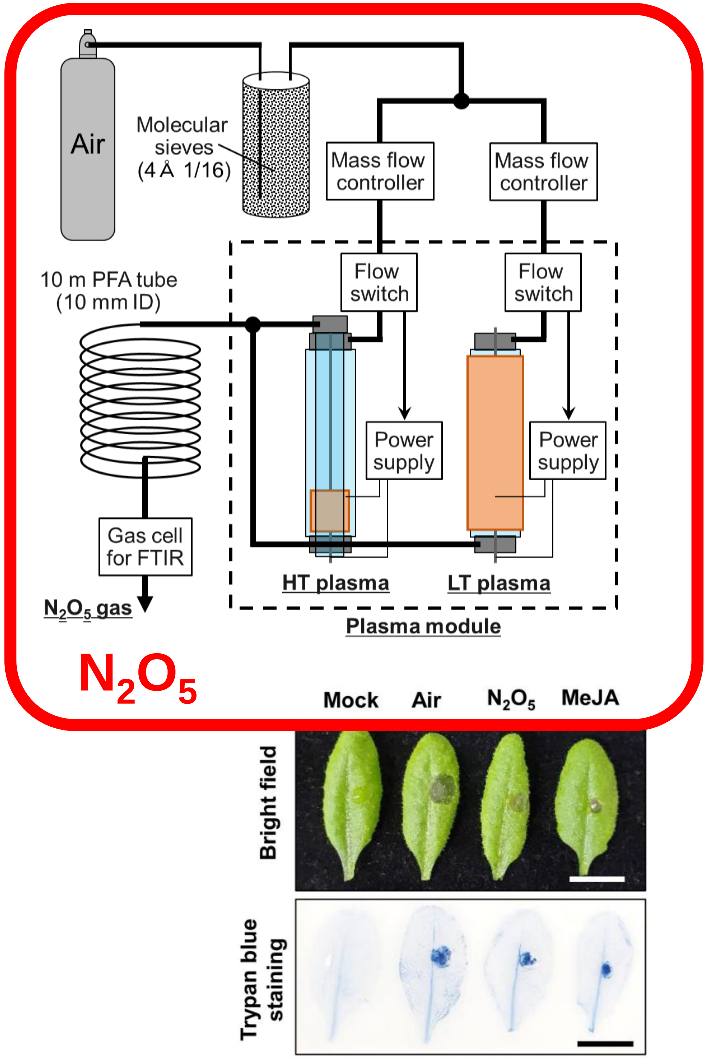
Air APP Irradiation System : Agriculture Applications

【sterilization/virus inactivation】 (during cultivation)





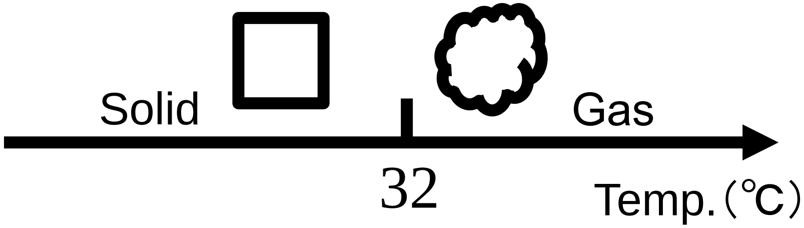
【plant immunity activation】 【nitrogen fertilization】

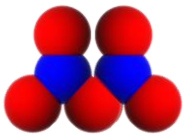


T. Kaneko, K. Ishikawa, et al., Jpn. J. Appl. Phys. **61**, SA0805 (2022)

Dinitrogen Pentoxide (N_2O_5)

Properties





Molecular weight
108.01

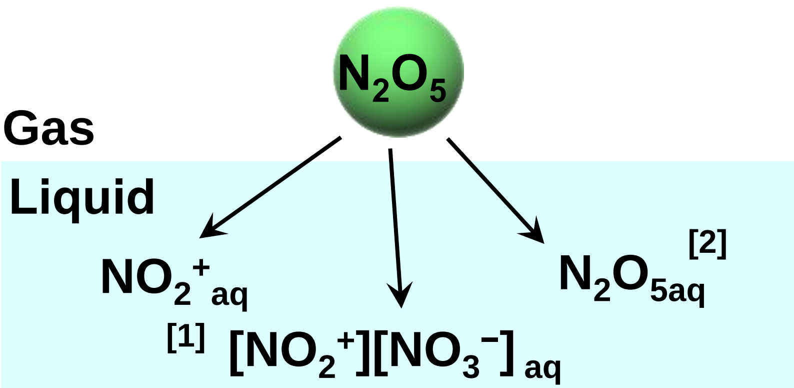
Preparation

$$P_4O_{10} + 12HNO_3 \rightarrow 4H_3PO_4 + 6N_2O_5$$

- ✓ Hazardous
- ✓ Concentrated acid -> Environmental pollution

Unique reactivity

At gas-liquid interface



● Reactive intermediate form

$NO_2^+{}_{aq}$, $[NO_2^+][NO_3^-]_{aq}$, $N_{2}O_{5aq}$

- $+ H_2O$ → NO_3^-
- Others → Oxidation, Nitration

[1] A.F. Holleman: Inorganic chemistry (De Gruyter, Berlin, 2001).
[2] M. Galib and D.T. Limmer, Science (80-.). **371** (2021) 921.

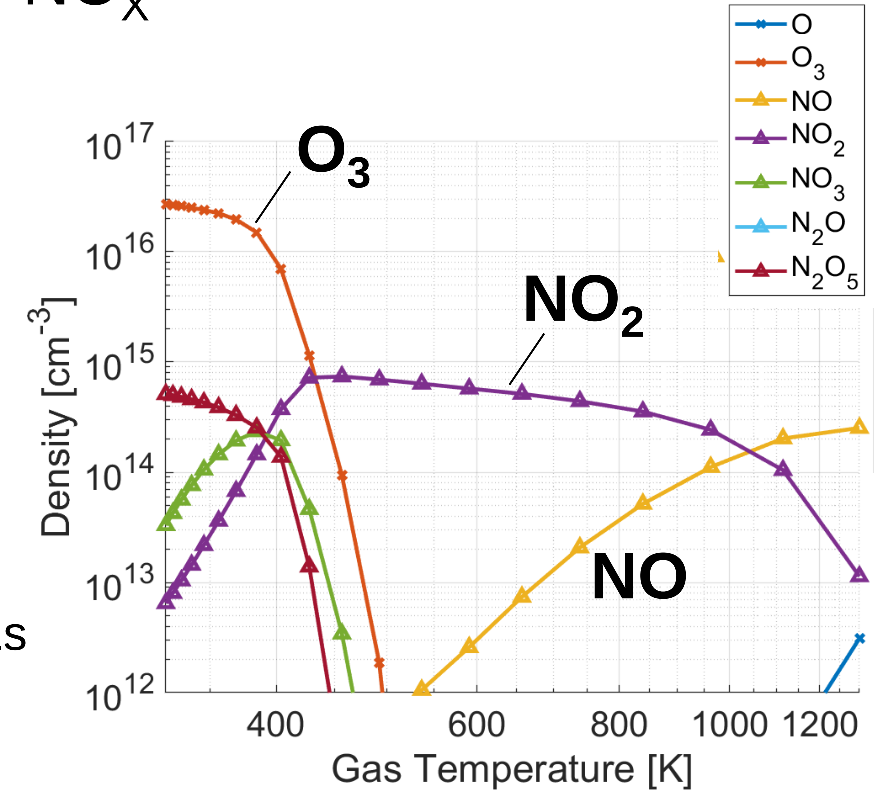
How to Synthesize N_2O_5 Selectively ?

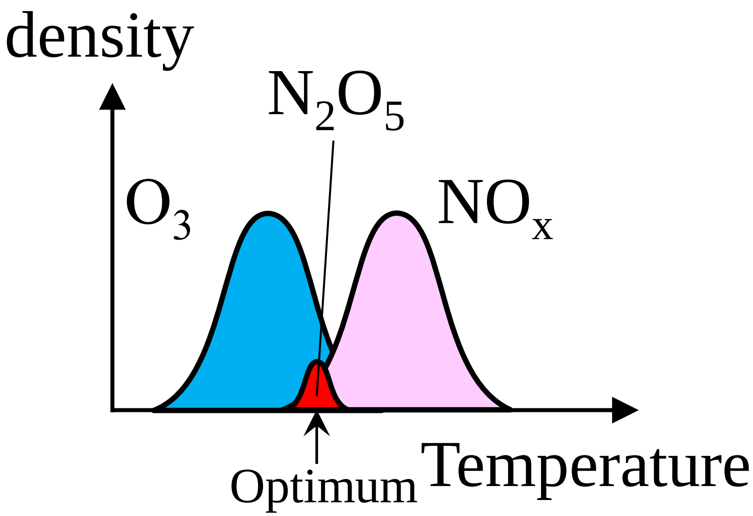
Balanced Generation of O_3 and NO_X

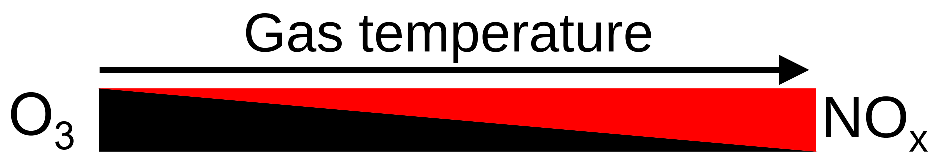
$$O_3 + 2NO_2 \rightarrow N_2O_5 + O_2$$

Radical	Flux (molecules/cm³/s)
N	0.5×10^{17}
O	1.3×10^{17}

Temperature: 298 K
Reaction time after plasma region : ~ 1s





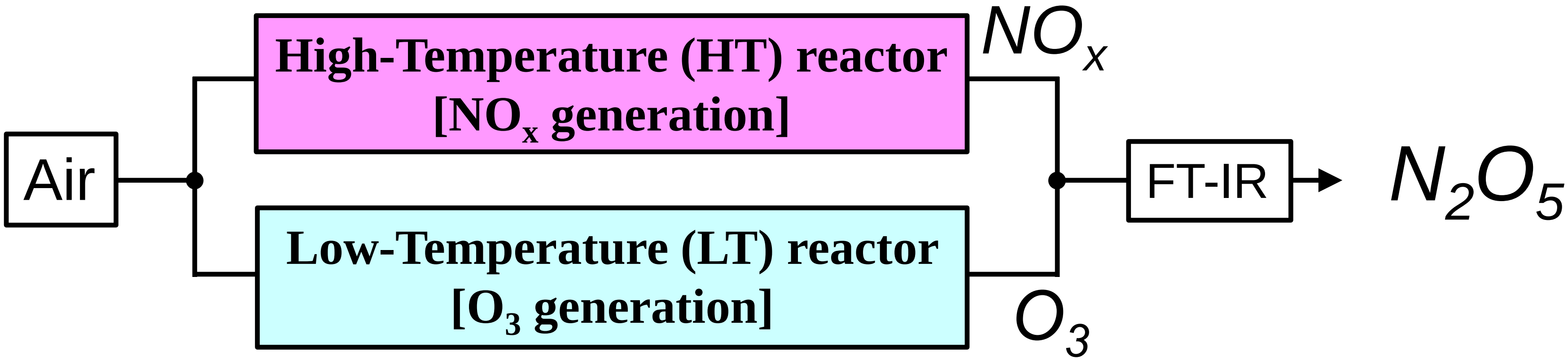


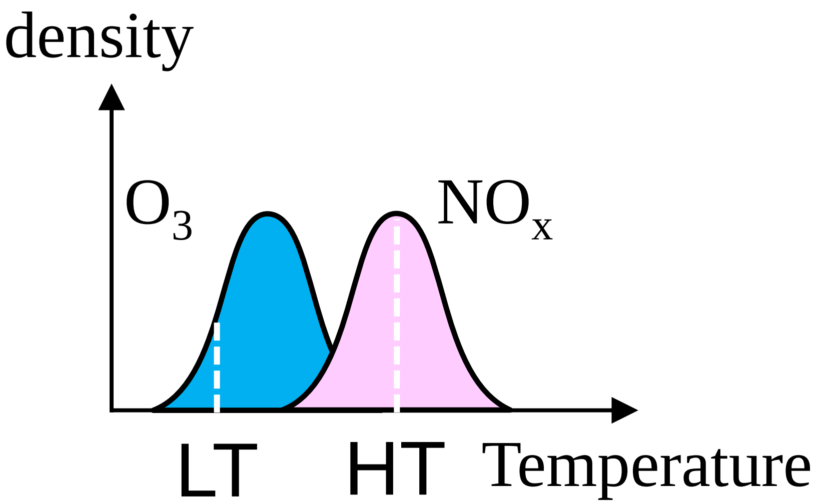
Balanced generation of O_3 and NO_x is difficult using single plasma reactor

Simple Idea for Selective N_2O_5 Generation

Is it possible to generate N_2O_5 selectively ???

➡ Combination use of O_3 generator and NO_x generator

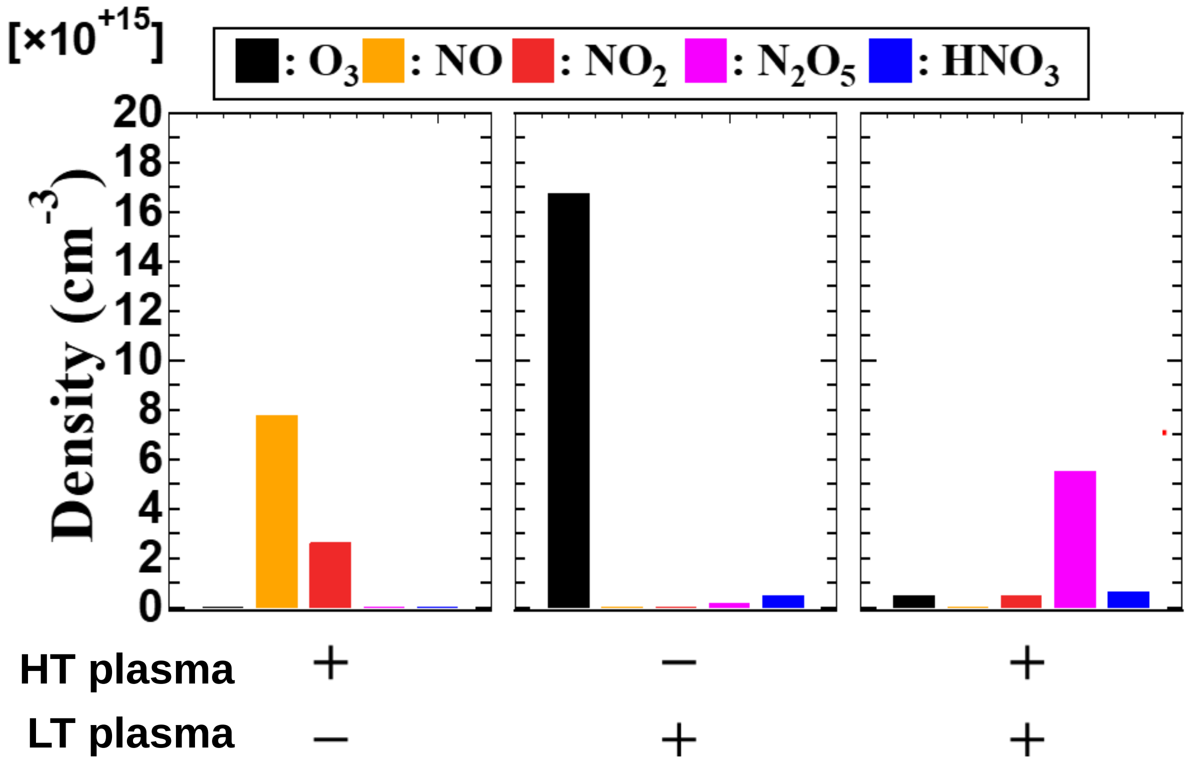


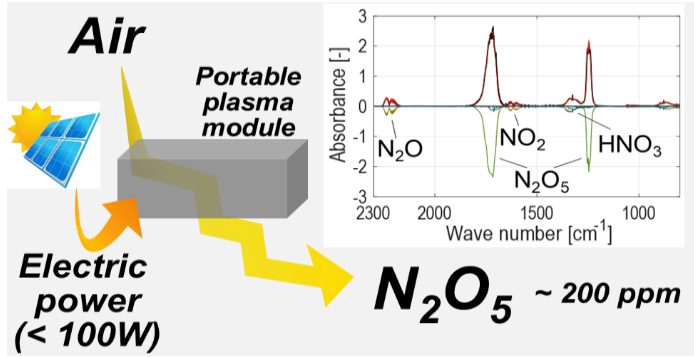


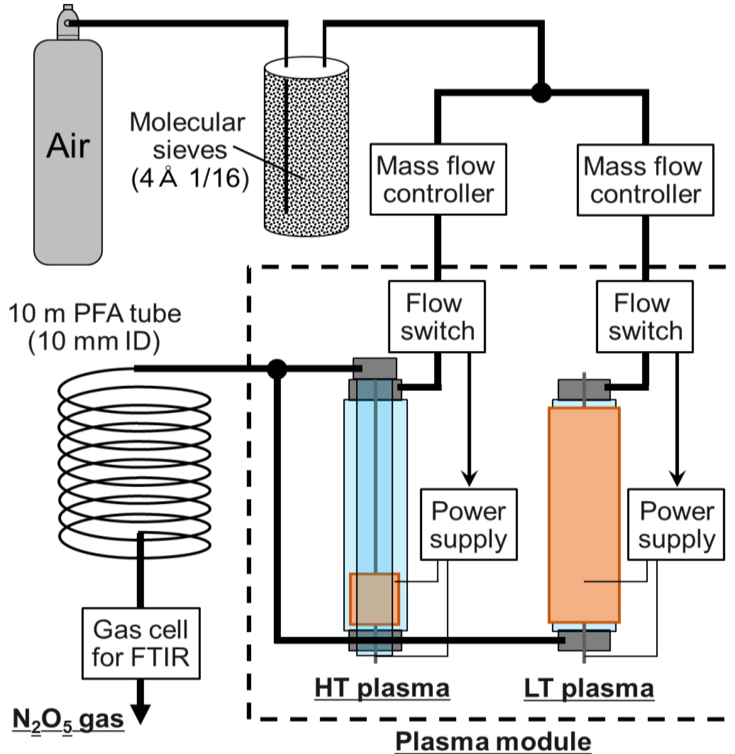
$$\left[O_3 + NO \rightarrow O_2 + NO_2\right]$$

$$O_3 + 2NO_2 \rightarrow N_2O_5 + O_2$$

High Density N_2O_5 Production from Air Plasma

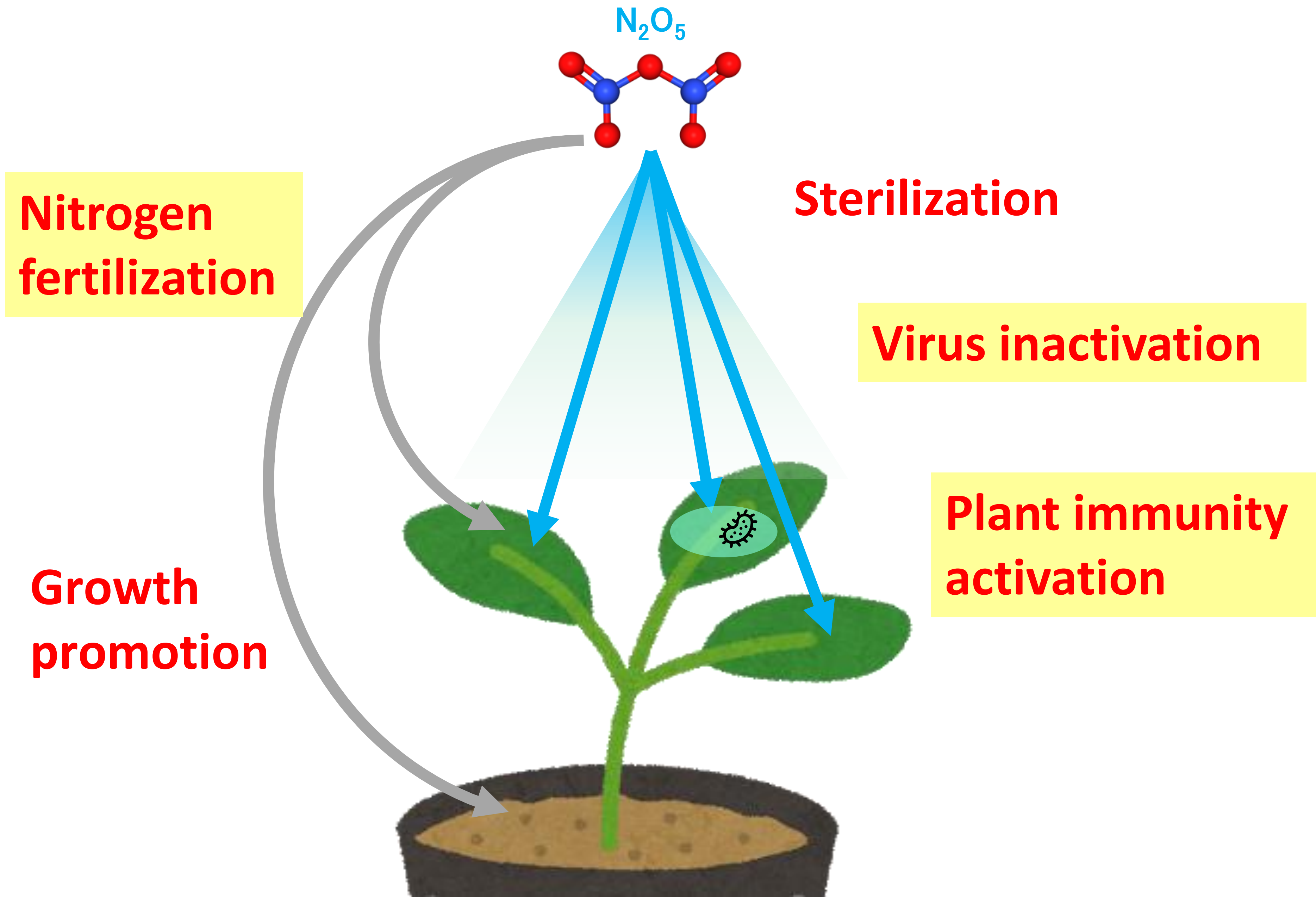






S. Sasaki, K. Takashima, and T. Kaneko: Ind. Eng. Chem. Res. **60**, 798 (2021)

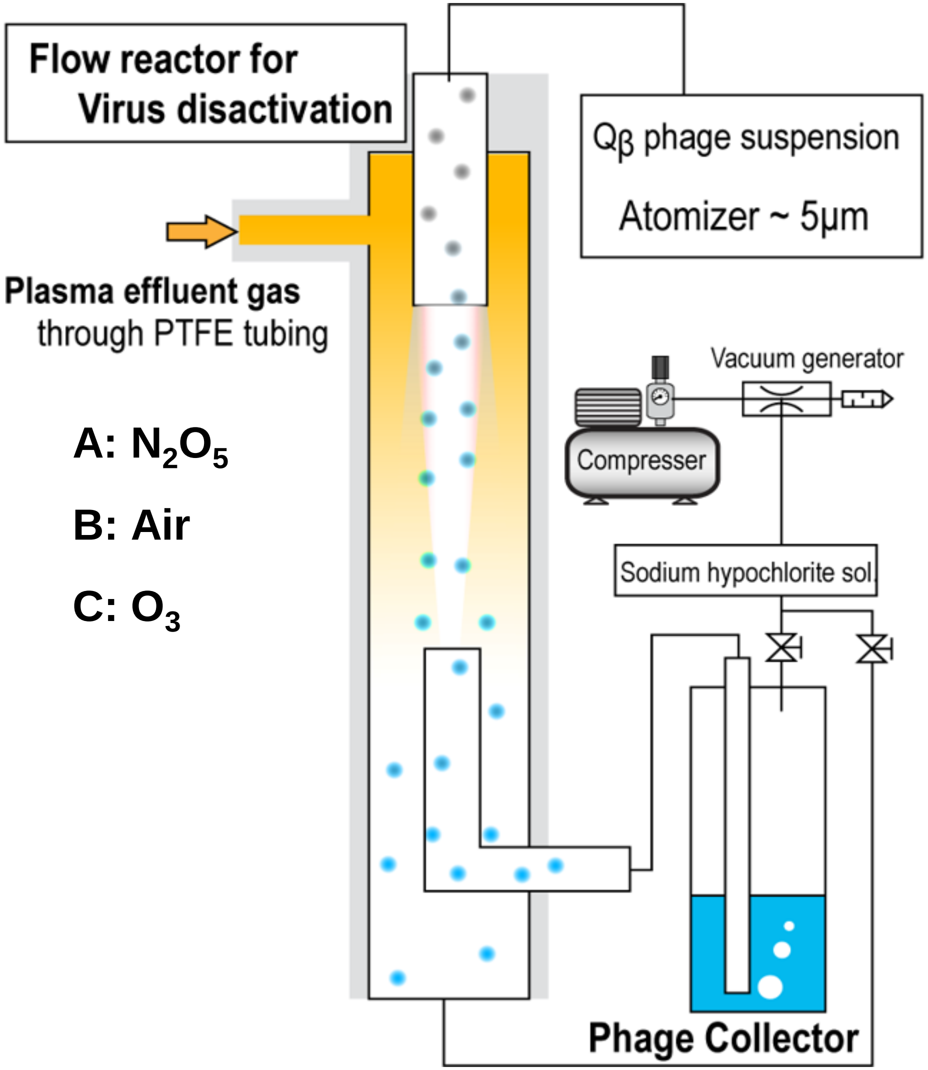
Potential Effects of N_2O_5 Exposure to Plants



Applications of Air APP-N_2O_5

- Qβ phage Virus Inactivation Effects
- Nitrogen Fertilization Effects
- Activation of Plant Immunity

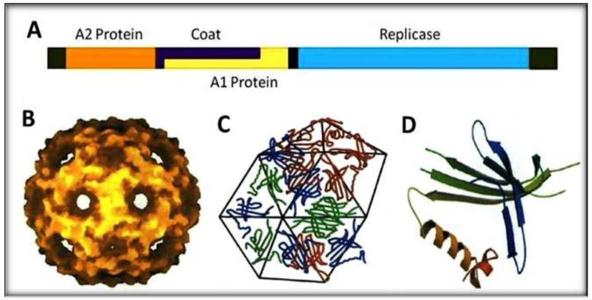
Virus Inactivation Effects by APP-N_2O_5



A: N_2O_5

B: Air

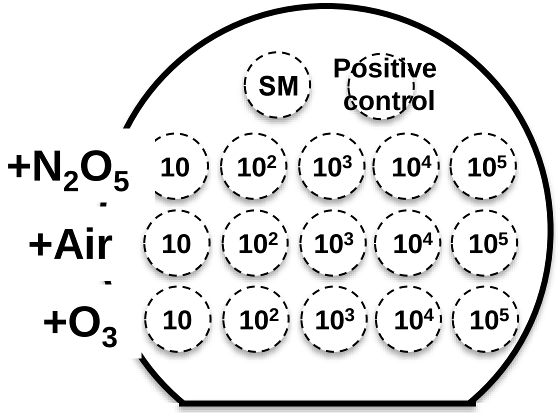
C: O_3

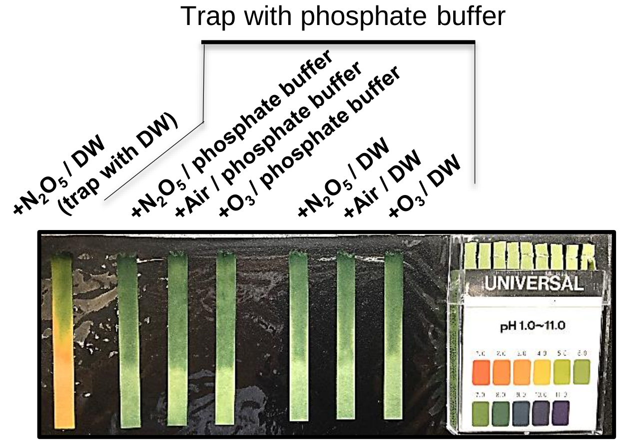


Qβ phage virus infects bacteria such as E. coli.

Plaque Assay: Virus Inactivation Using APP-N_2O_5

Mist of Qβ phage (1x10^9 pfu/ml)
→ N_2O_5 treatment for 15 min
→ Trap with 30 ml of 100mM phosphate buffer (pH7.0)
→ Plaque assay

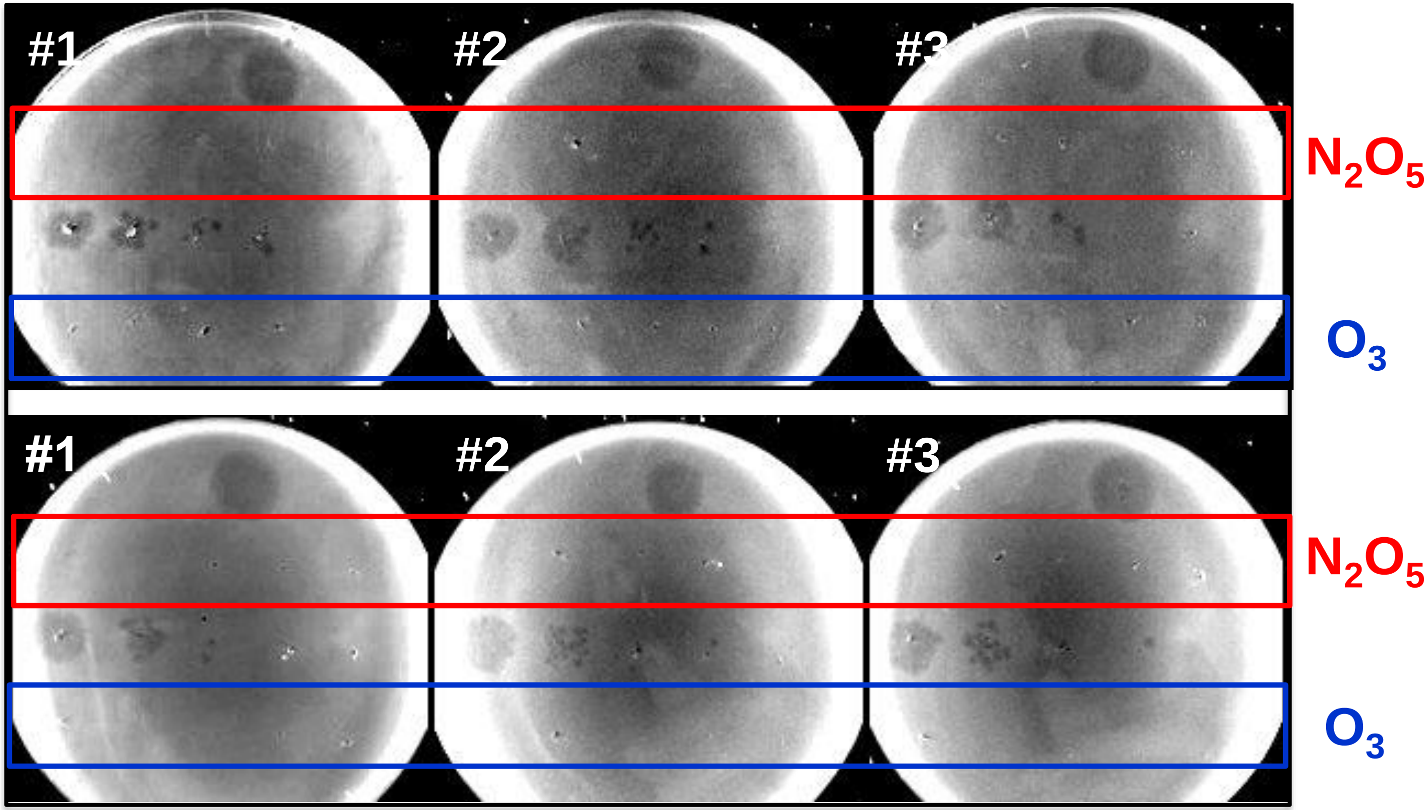




Mist solution: DW

> 3-log reduction in titer of Qβ phage

Mist solution: phosphate buffer (pH7.0)



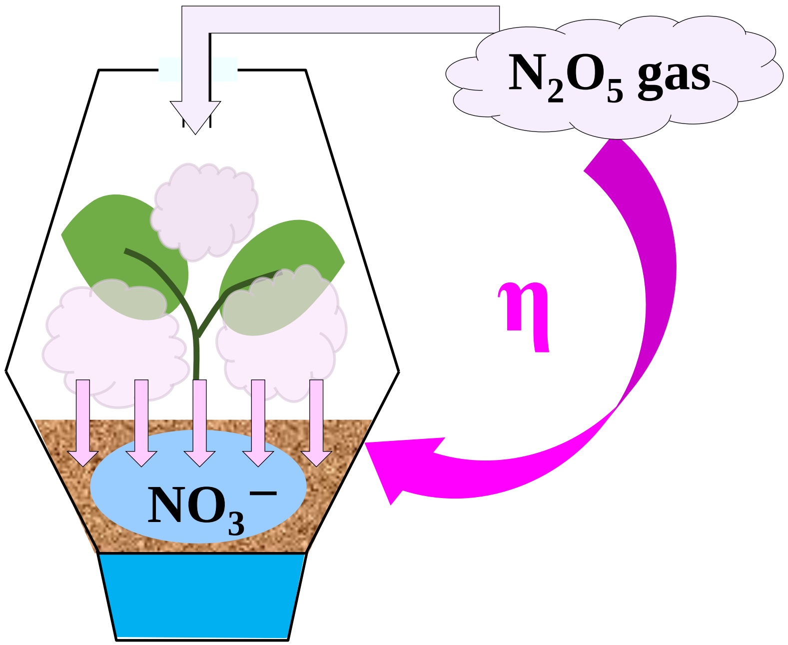
TOHOKU UNIVERSITY

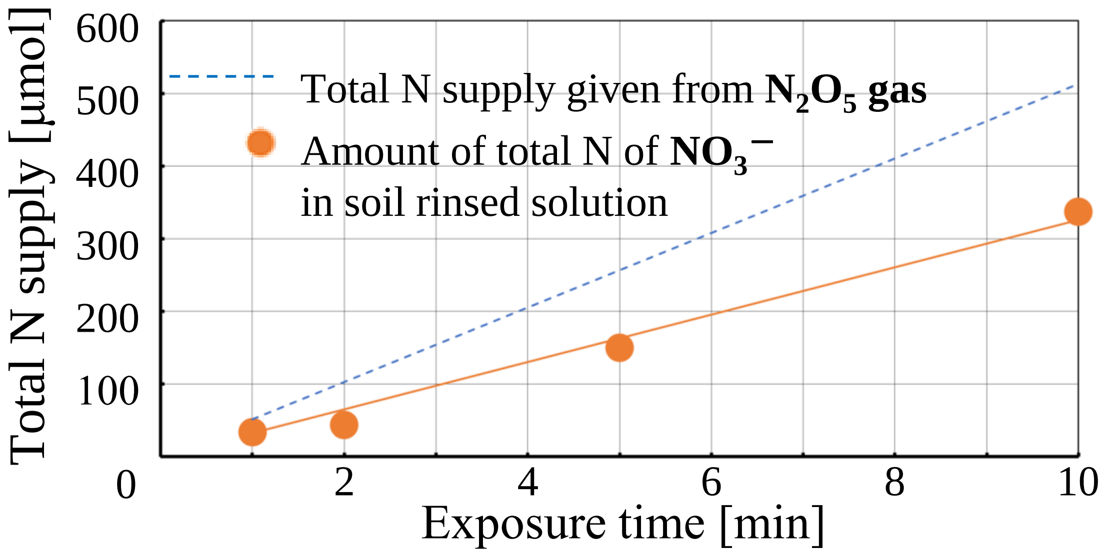
Applications of Air APP-N_2O_5

- Qβ phage Virus Inactivation Effects
- Nitrogen Fertilization Effects
- Activation of Plant Immunity

Conversion Efficiency From N_2O_5 to Nitrate

Direct Exposure



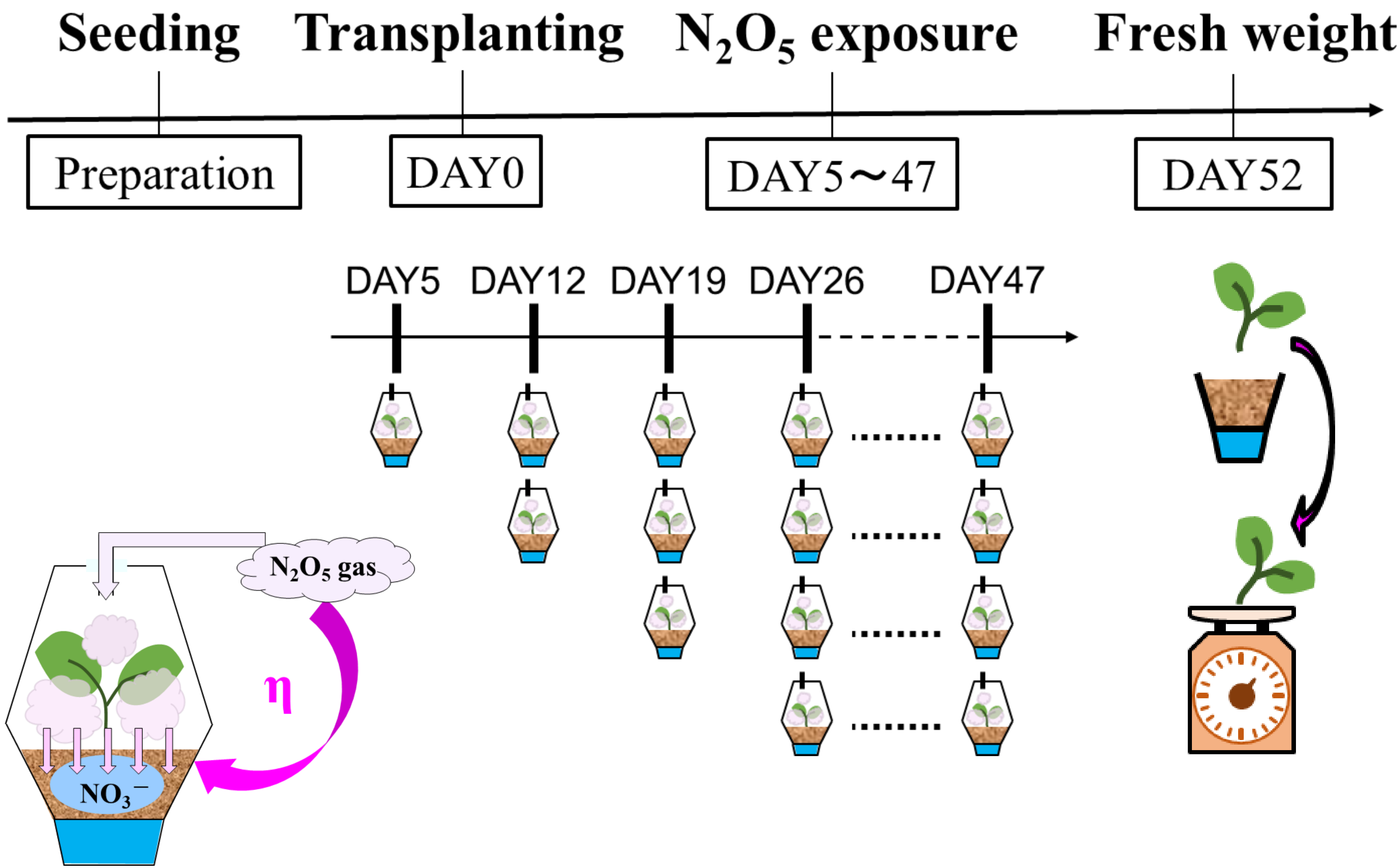


η=63%

η: Conversion efficiency

High efficiency nitrogen fertilization !

N_2O_5 Fertilization Methods

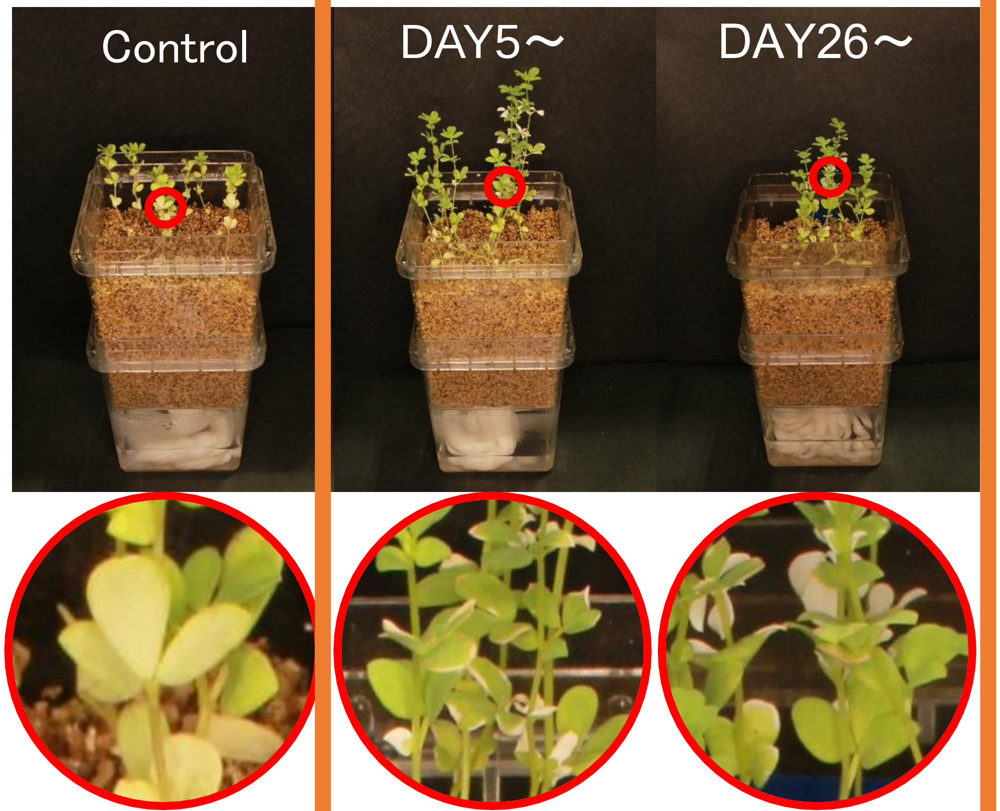


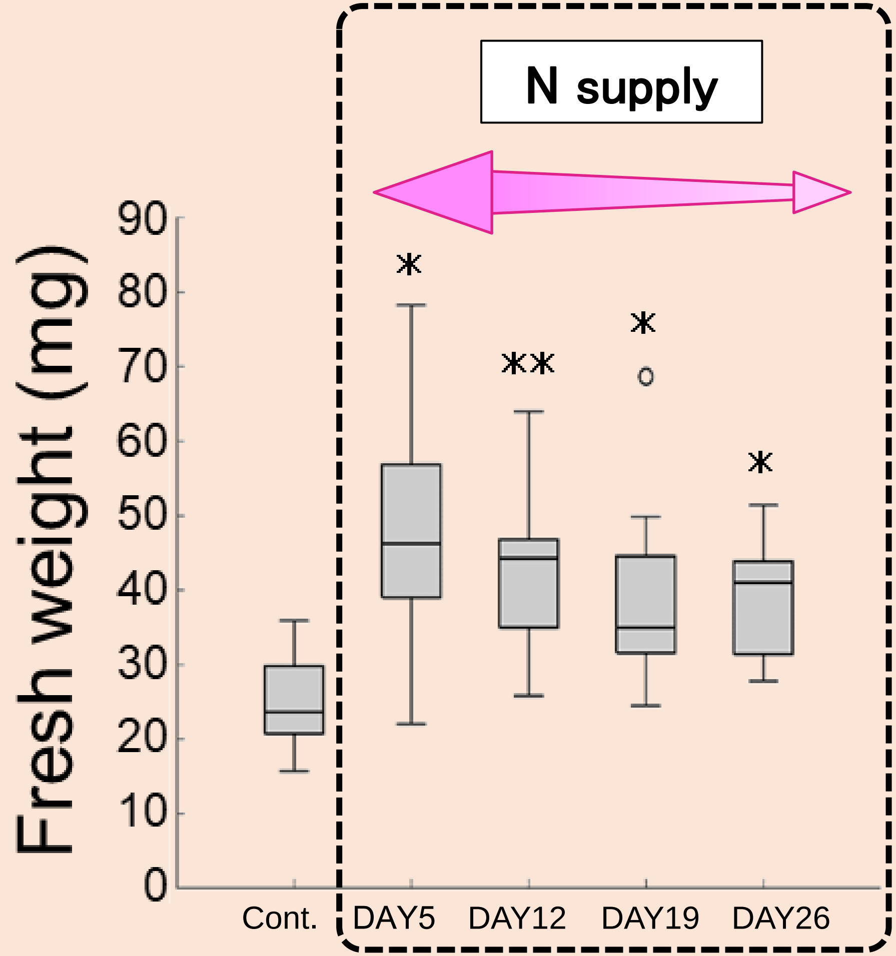
N_2O_5 Fertilization Effects on Plant Growth

Nitrogen deficient *Lotus japonicus*

30s exposure

N_2O_5 Exposure Start Day





N_2O_5 Exposure Start Day

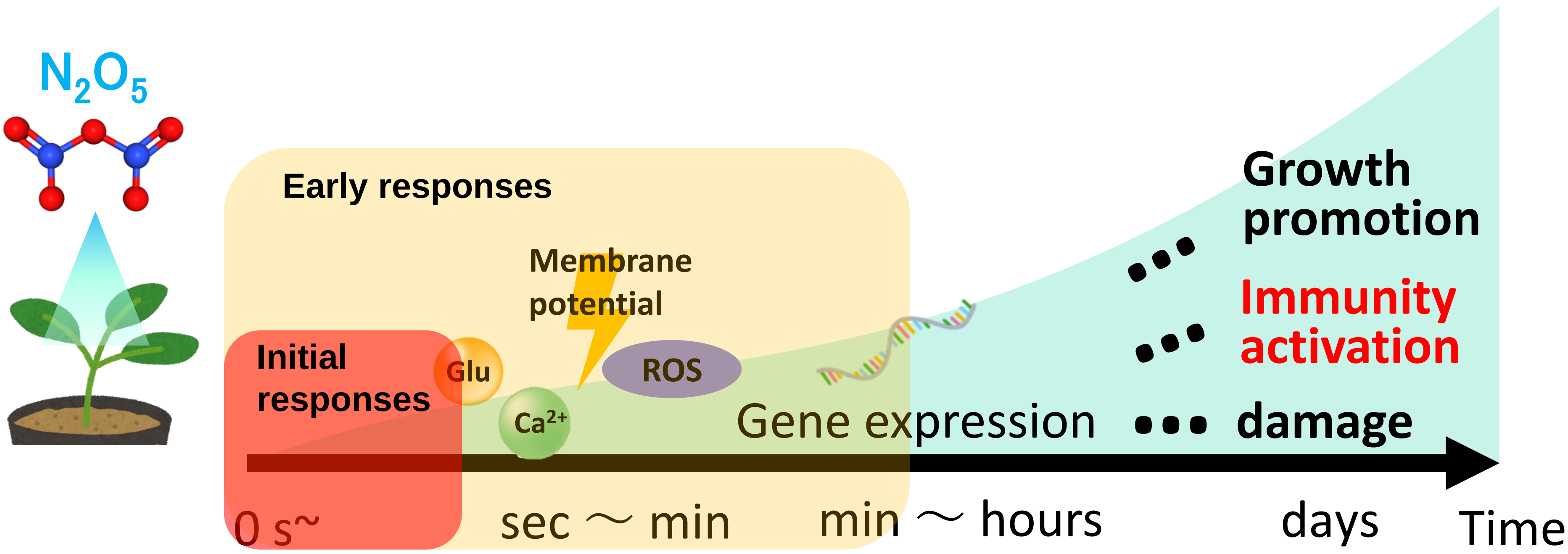
Observation: DAY47

Observation: DAY52

Applications of Air APP-N_2O_5

- Qβ phage Virus Inactivation Effects
- Nitrogen Fertilization Effects
- Activation of Plant Immunity

Investigation on Action Mechanism of N_2O_5 to Plants



- **Initial responses**
  Ca^{2+} signaling
- **Early responses**
  Gene expression

Knowledge in plant biology

Relation between stimulus and responses

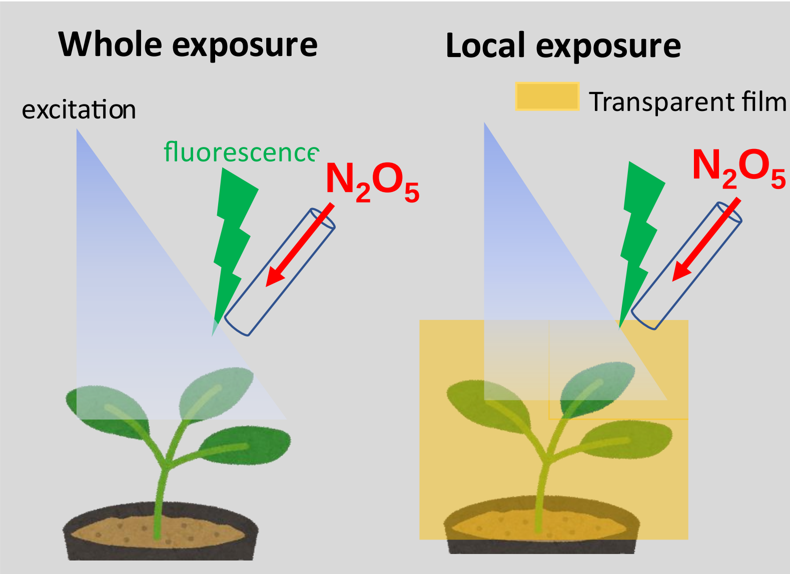
- Initial responses to gene expression
- gene expression to phenotype
  etc…

✓ Prediction of N_2O_5 effects on plants

✓ Proposal of agricultural applications using N_2O_5

Experimental Method

Live imaging of $[Ca^{2+}]_{cyt}$



Arabidopsis Thaliana expressing a calcium ion sensor protein (GCaMP3)

Measurement of gene expression

Focus on **plant immunity**

sampling

RT-qPCR

Since exposure

0 m — 10 m — 24 h

Live imaging of Ca^{2+} signaling → Sampling for PCR → RT−qPCR

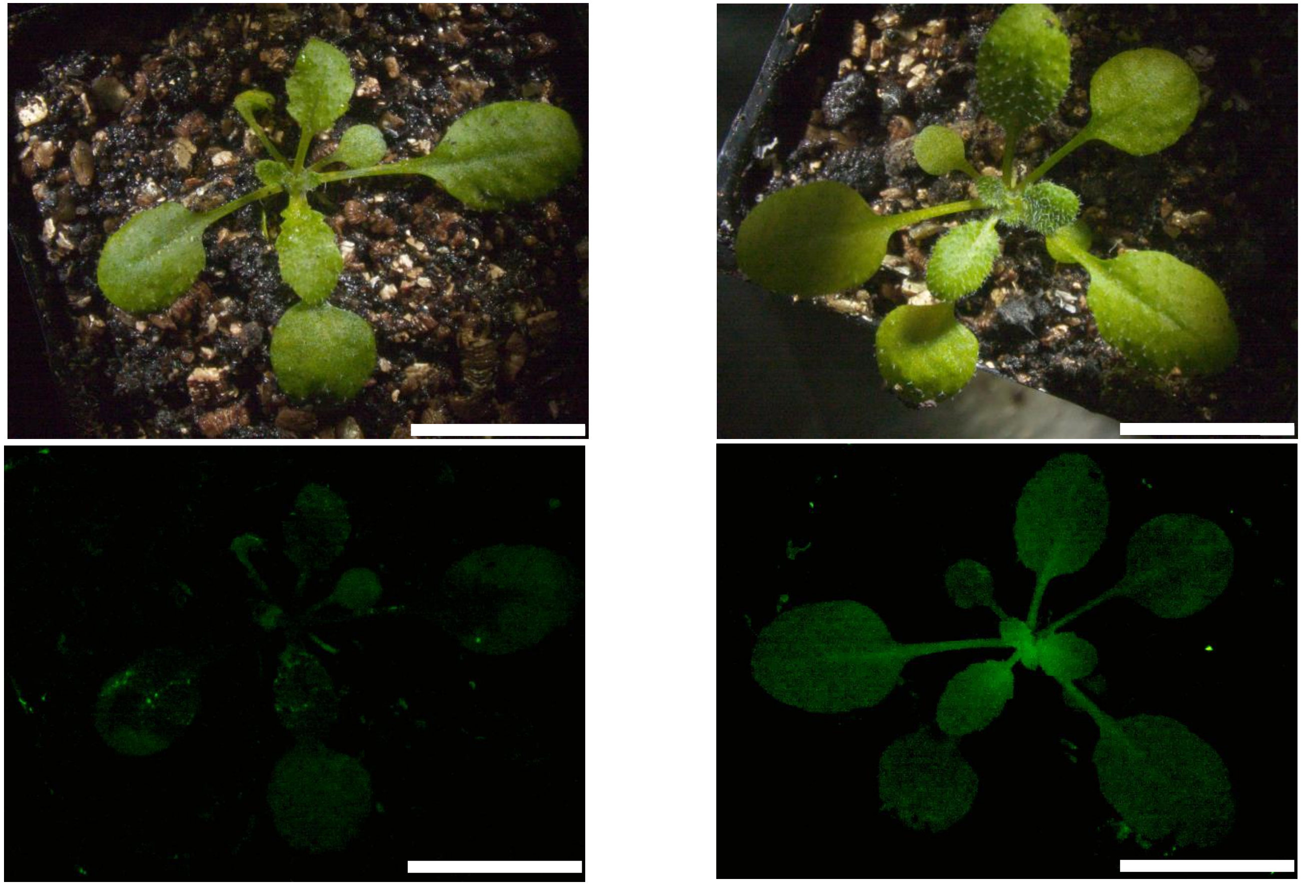
- Inactivate the cellular activity
- homogenization
- RNA extraction
- cDNA generation

- JA-related genes
- Antimicrobial peptide-related gene

Live Imaging of Ca^{2+} Response after Whole Exposure

Air | **N_2O_5**

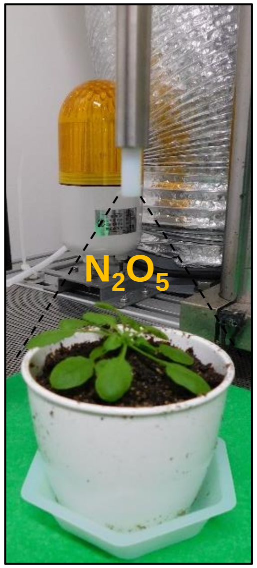
Pre exposure / Since exposure



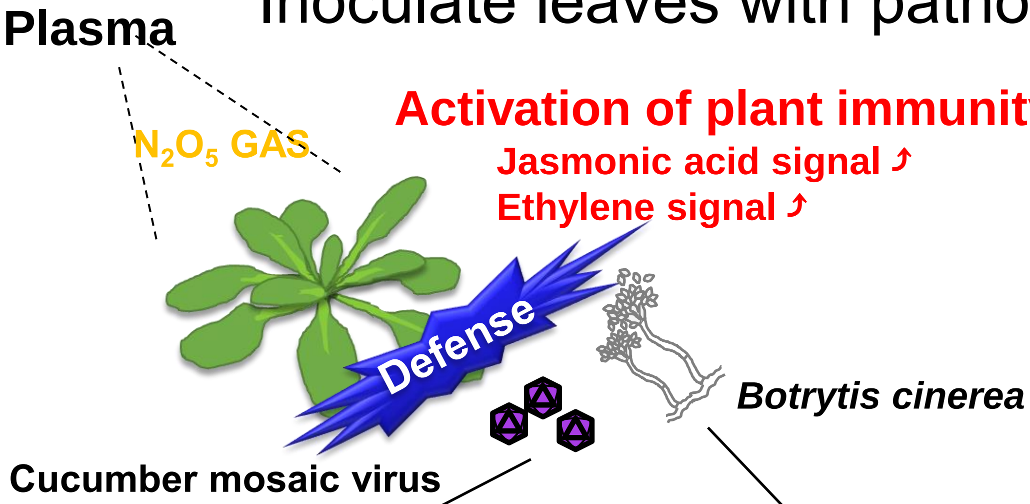
N_2O_5 : 0.3 [μmol/s]
exposure time : 10 [s]
Scale bar : 1 [cm]

- Air : No $[Ca^{2+}]_{cyt}$ increase
- N_2O_5 : **Increase** $[Ca^{2+}]_{cyt}$ ➡ **Decrease** over time
  - ☐ stimulated on plant surface
  - ☐ through stoma etc…

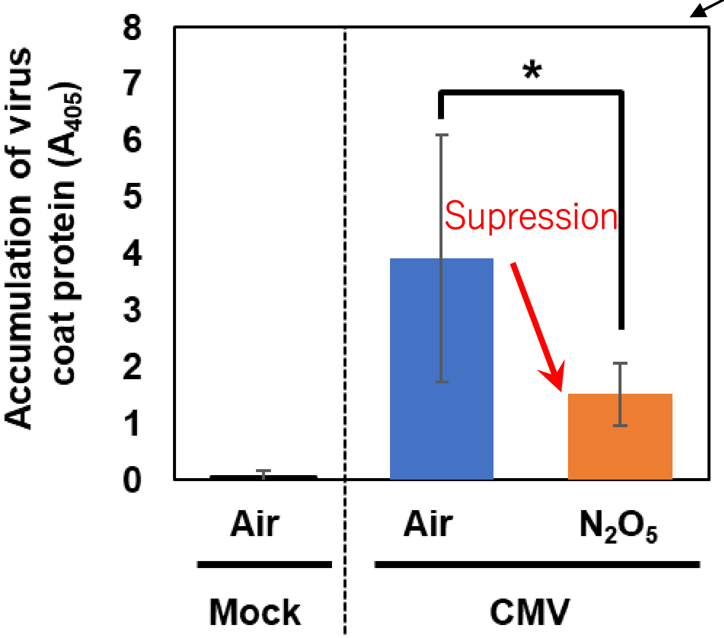
Activation of Plant Immunity

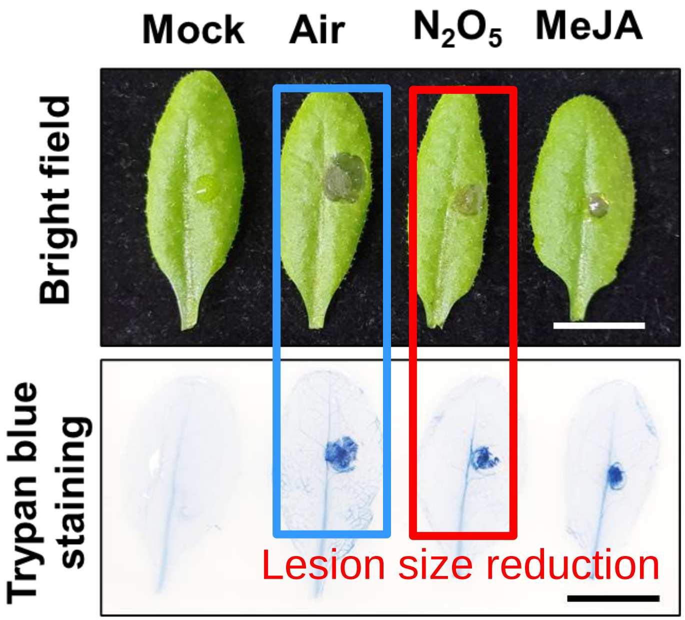


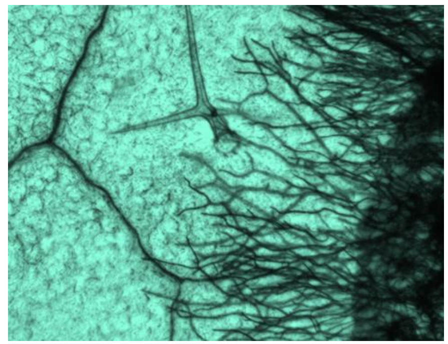
Inoculate leaves with pathogen



Immunity activation effect of N_2O_5 was demonstrated by the inoculation tests







B. Cinerea **hyphae infecting to leaf tissue**

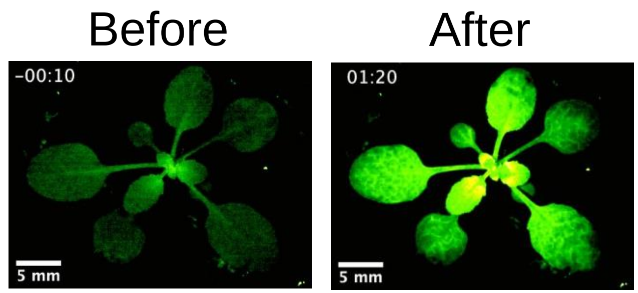
D. Tsukidate, K. Takashima, S. Sasaki, S. Miyashita, T. Kaneko, H. Takahashi, and S. Ando: PLOS ONE **17**, e0269863 (2022).

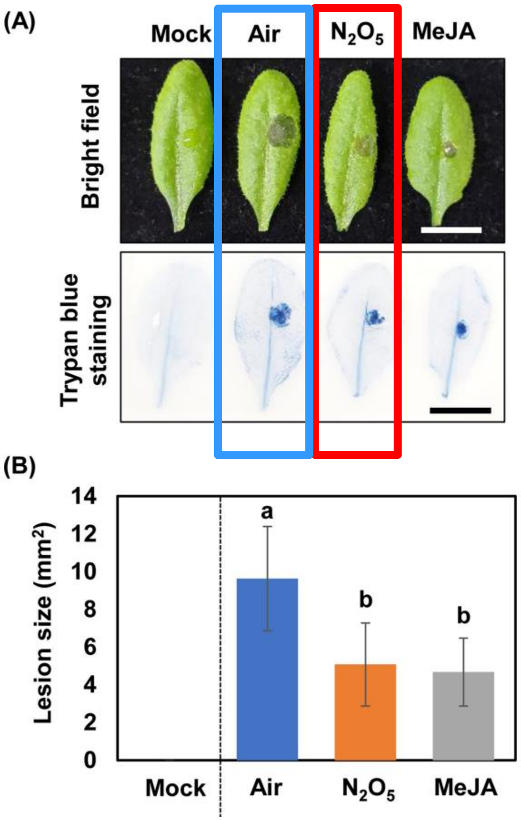
Activation of Plant Immunity by Air APP-N_2O_5

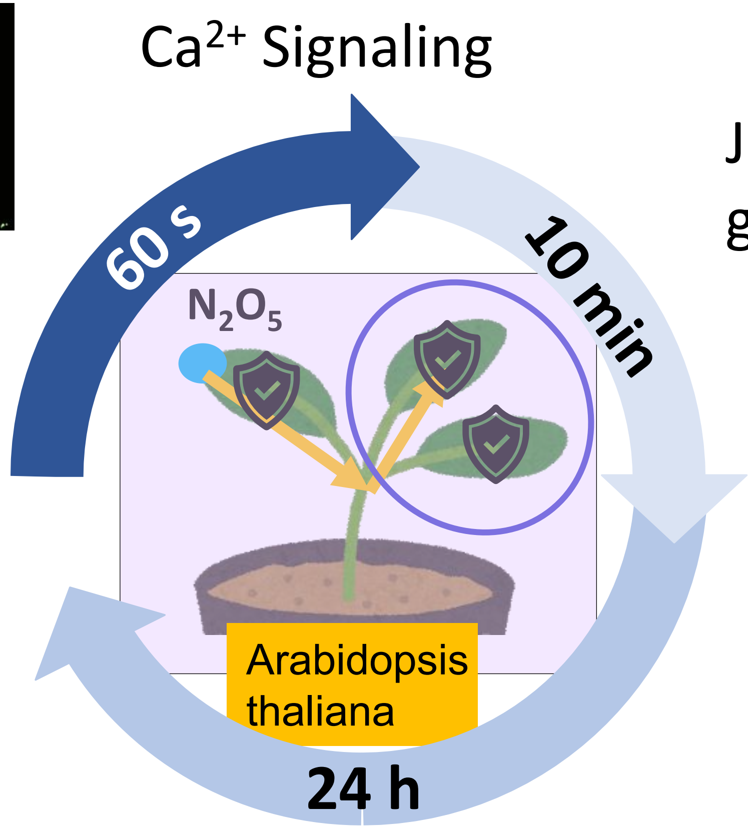
Ca^{2+} Signaling by N_2O_5 exposure

$[Ca^{2+}]_{cyt}$

Low — High







Jasmonic acid-related gene expression

Expression of genes encoding antimicrobial peptides

Activation of Plant Immunity

D. Tsukidate, K. Takashima, S. Sasaki, S. Miyashita, T Kaneko, H. Takahashi, S. Ando, PLOS ONE **17,** e0269863 (2022) .

Summary

We have developed a new air atmospheric pressure plasma device synthesizing high density N_2O_5 selectively and demonstrated the applications for sterilization, fertilization, and plant immunity activation.