Effect of Ethylene and Carbon Dioxide on Seed Germination of *Monochoria vaginalis* var. *plantaginea**

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Abstract: The relationship between seed germination of *Monochoria vaginalis* var. *plantaginea* and gas composition in soil, i.e. ethylene, CO₂, O₂ and their combination was studied. A high germination percentage was obtained in the presence of both ethylene and CO₂ regardless of the storage conditions, but in the presence of absorbents for both ethylene and CO₂ the germination percentage declined significantly (Fig. 2 and Table 1). In the presence of CO₂ absorbent, the application of ethylene to seed increased the germination percentage, while the effect of exogenous CO₂ with the absorbent of ethylene on seed germination was very little. However, the germination percentage was increased by exogenous CO₂ if the concentration was sufficiently high (Table 1). The effect of ethylene on seed germination of *Monochoria* may more important than that of CO₂, and the seed germination is considered to be increased by endogenous ethylene.

The seeds under 60% oxygen exhibited a significantly decreased germination percentage while under 0.2% oxygen they showed high germination. No seeds, even non-dormant seeds, germinated in a petri dish with a filter paper in the presence of 21% oxygen (Fig. 1). The results demonstrate that *Monochoria* seeds require very little oxygen for germination.

Key words: C₂H₄, CO₂, germination, *Monochoria*

seeds, O₂

Introduction

Monochoria vaginalis (Burm. f.) Presl var. *plantaginea* (Roxb.) Salms-Laub (hereafter *Monochoria*) is a typical hydrophytic, annual broad-leaved weed. The increase in annual paddy weeds due to a shifting herbicide resistance of weeds and careless water control in paddy fields has recently become an issue¹⁶⁾. The mechanism of germination and dormancy of *Monochoria* is still little understood. An earlier paper by Chisaka and Kataoka¹⁾ reported that a high germination percentage of *Monochoria* was obtained from seeds illuminated by light, incubated in a glass container sealed with a rubber stopper to maintain low oxygen (below 1%), and exposed to ethylene (10 ppm). Also, Kataoka and Kim⁹⁾ reported that *Monochoria* seeds showed higher germination rate under low oxygen partial pressure than in the atmosphere. In fact, *Monochoria* seeds have been observed to germinate well in flooded paddy fields. It is known that exogenous ethylene or CO₂ stimulates growth of both axes and cotyledons in seeds²⁾, and one action of CO₂ in breaking dormancy of the seeds is their growth promotion by stimulating ethylene production⁴⁾. In many other species, the presence of CO₂ during the early stages of imbibition of dormant seeds was necessary for the ethylene action^{3,4,13~15)}. Jackson⁷⁾ suggests that endogenously produced ethylene may be supplemented by environmental ethylene produced in flooded soil. Flooded soil produces most ethylene when the supply of oxygen is restricted to 1 KPa or less¹⁷⁾, and there is an

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increasing rate of ethylene production with temperature (5–65°C) in soil with a high organic matter content⁵⁾. In well-aerated soil, on the other hand, ethylene is metabolized or absorbed by grampositive mycobacteria⁷⁾. Thus, the balance between formation and degradation would determine how much dissolved ethylene is present in the soil. In these circumstances, seed germination may be affected by gaseous condition of the paddy field.

In this study, we investigated the relationship between seed germination of *Monochoria* and gas phase composition, i.e. ethylene, CO₂ and their combination.

Materials and Methods

Seed materials

Seeds collected at the experimental paddy fields of the National Institute of Agricultural Science, Konosu from the end of September through the end of October in 1978 were carefully selected and stored under different conditions according to the purpose of the experiment at the beginning of November. Most experiments were carried out from 1978 through 1980 at the National Institute of Agricultural Science.

Germination test

In all germination tests, 100 seeds were placed in each petri dish or glass tube. Seed germination was carried out under 3,000 lux of light at 30°C for 5–6 days. The germination test was repeated 2 or 3 times in each experiment.

Determination of C₂H₄, CO₂ and O₂

The concentration of ethylene was determined by a gas chromatograph equipped with a flame ionization detector (FID). The glass column (2 m×0.3 mm i.d.) was packed with Porapak Q. Carrier gas was N₂ (40 ml/min), and temperatures were 100°C for injection and 80°C for column. Concentrations of CO₂ and oxygen were detected by a gas chromatograph equipped with a thermal conductivity detector (TCD). The stainless steel column was 1 m×0.3 mm (i.d.) packed with either

molecular sieves 5A for CO₂ or Porapak Q for oxygen. Carrier gas for both gases was He (40 ml/min), temperatures were at 70°C for injection and detector, and the column was at 50°C. One milliliter of gas sample was injected for all gas determinations.

Experiment I: Effect of storage on seed germination

The experiment was carried out to investigate the effects of seed storage and the time elapsed after harvest on seed germination under various seed beds. Seeds were stored under 3 different conditions as follows: (1) Dry condition at 5°C (seeds were stored in a desiccator which was placed in a 5°C-cold room as the control); (2) Moist condition at 5°C (seeds were stored in a covered petri dish on moist filter paper which was placed in a 5°C-cold room); (3) Field condition (the bottom of a Wagner pot (1/10,000 a) was removed and covered with a fine netted nylon cloth. Seeds were mixed with soil sieved through a fine mesh and the mixture was placed on the netted nylon cloth bottom and covered with soil to a depth of 2–3 cm. The soil that was mixed with seeds had been treated at 50–60°C for 48 h to kill other kinds of seeds. The pot was then buried in a paddy field).

Seed germination was tested using 4 methods. (A) Seeds were germinated in a petri dish on moist filter paper; (B) Seeds were placed in a 55 ml-glass tube (7.5 cm in height) with water to a depth of 1 cm; (C) Seeds were placed in a 55 ml-glass tube with a wet filter paper on the bottom and filled with nitrogen (99.6% N₂) instead of air; (D) Seeds were placed on the surface of 1 cm of water-saturated soil in a 55 ml-glass tube. The glass tube used for the germination test was sealed with a rubber stopper.

Experiment II: Effects of storage and gas phase composition on seed germination

The experiment was conducted to determine the effects of storage and gas combination of oxygen, ethylene or CO₂ on germination. Seeds

stored under dry and moist conditions at 5°C as described previously for about 2 months were used in this experiment. They were placed in a 55-ml-glass tube with wet filter paper on the bottom for germination. The gas composition was adjusted as follows: A-1) Ambient air (21% O₂) with and without absorbents of ethylene and CO₂ were used for the controls. The absorbents used were 0.25 M Hg(ClO₄)₂ for ethylene and 20% KOH solution for CO₂. A-2) In the presence of CO₂ absorbent, air plus 50 ppm ethylene. A-3) In the presence of ethylene absorbent, air plus 5% CO₂. A-4) Without absorbents, air plus 50 ppm ethylene and 5% CO₂. In air with absorbents, concentrations of ethylene and CO₂ measured were 0 ppm and 0%. In air without absorbents, the concentrations of ethylene and CO₂ measured were 0.08 ± 0.01 ppm (mean of 3 experiments $\pm$ SE) and $0.2 \pm 0.04\%$, respectively. Injected 50 ppm ethylene and 5% CO₂ measured were 50 ± 0.5 ppm and $5 \pm 0.2\%$. B-1) A zero percent oxygen environment with and without absorbents of ethylene and CO₂ was generated by replacing air with nitrogen. B-2) In the presence of CO₂ absorbent, 0% oxygen plus 50 ppm ethylene. B-3) In the presence of ethylene absorbent, 0% oxygen plus 5% CO₂. B-4) Without absorbents, 0% oxygen plus 50 ppm ethylene and 5% CO₂. In zero percent oxygen, concentration of oxygen measured was 0.2% O₂ (99.8% N₂). Concentrations of injected 50 ppm ethylene and 5% CO₂ measured were 50 ± 0.3 ppm and $4 \pm 0.2\%$. C-1) Sixty percent of oxygen with and without absorbents of ethylene and CO₂. Oxygen was mixed with air to achieve a gas phase of 60% oxygen. C-2) In the presence of CO₂ absorbent, 60% oxygen plus 50 ppm ethylene. C-3) In the presence of ethylene absorbent, 60% oxygen plus 5% CO₂. C-4) Without absorbents, 60% oxygen plus 50 ppm ethylene and 5% CO₂. Injected 60% oxygen measured was $60 \pm 2\%$ O₂. Injected 50 ppm ethylene and 5% CO₂ measured were 50 ± 0.6 ppm C₂H₄ and $4 \pm 0.4\%$ CO₂. After gas was injected into

the sealed glass tube containing seeds, all gas concentrations were immediately checked. A small glass tube containing wet filter paper with an absorbent solution of ethylene or CO₂ was placed in a 55 ml-glass tube.

Experiment III: Effects of gas concentrations of ethylene and carbon dioxide on seed germination

To study the effects of seed storage and gas concentration of ethylene and CO₂ on seed germination, seeds stored under dry and moist conditions at 5°C for about 2 months were used. They were placed on a dampened filter paper raised like a platform using a glass container which was placed upside down in a 55 ml-glass tube for germination. In all glass tubes, ambient air (21% O₂) was the concentration of oxygen. Injected ethylene concentrations measured were 0.7 ± 0.05 ppm (mean of 2 experiments $\pm$ SE), 8 ± 0.5 ppm and 35 ± 2 ppm, respectively. Gas combination was paired with each ethylene concentration in all CO₂ concentrations. Injected CO₂ concentrations measured were $0.1 \pm 0.05\%$ (mean of 2 experiments $\pm$ SE), $0.3 \pm 0.05\%$, $1 \pm 0.15\%$ and $4 \pm 0.35\%$, respectively. Ambient air with and without the two absorbents of ethylene and CO₂ was used as the control. After gas was injected into the sealed 55 ml-glass tube containing seeds, all gas concentrations were immediately checked.

Results

Effect of seed storage conditions on seed germination

Effects of storage on seed germination are shown in Fig. 1. Seeds immediately after harvest did not germinate under any of the four germination conditions. Seeds 6 weeks after storage under a dry condition at 5°C showed germination of over 50%. On the other hand, over 70% of seed germination was found in seeds 2 weeks after storage under a moist condition at the same temperature and in an outdoor field.

No seeds from any of the storage conditions

Fig. 1. Changes in the germination percentage of *Monochoria* seeds during various periods under three different storage conditions.

Seed storage began on 1st November under three conditions. Germination was tested by the following 4 methods.

■, aerobic (air) condition; ○, flooding condition; □, anaerobic (N₂) condition; ●, soil condition.

See details of the storage and germination conditions in the text.

All glass tubes were sealed with a rubber stopper.

Each point is the mean of 3 experiments ($\pm$ SE).

germinated on wet filter paper with atmosphere as the air condition, while seeds from all the conditions showed a high germination percentage in the sealed glass tube with a low oxygen (0.2%) level. When stored under a dry condition under the gas phase replaced with nitrogen seeds, germination percentage increased dramatically compared to the other germination beds. Additionally, the seeds stored under any of the conditions did not germinate in the dark until about 4-

5 months after harvest (data not shown).

Effect of storage and gas phase composition on seed germination

Figure 2 shows the effect of ethylene and CO₂ in combination with various concentrations of oxygen on the germination of seeds stored under different conditions. All conditions of storage showed an increased germination under a low oxygen (0.2%) condition and a decrease in germination percentage under a high oxygen (60%) condition. There was a difference in seed response to ethylene and CO₂ depending on conditions of storage. In the control without the two absorbents of ethylene and CO₂, seeds stored under a dry condition at 5°C showed only 12% germination under an air (21% O₂) condition and 25% under low oxygen. While those stored under a moist condition at the same temperature showed 69% under air and 55% under 0.2% oxygen conditions. Seed germination was enhanced by exogenous ethylene and CO₂ under any oxygen concentration, even as high as 60%. However, in the presence of both the absorbents ethylene and CO₂, the germination percentage significantly declined. Gas combined with 50 ppm ethylene and 4 or 5% CO₂ under air caused an increased in germination of seeds stored under in dry and moist conditions at 5°C from 8% (0 ppm C₂H₄, 0% CO₂) to 68% and from 15% (0 ppm C₂H₄, 0% CO₂) to 83%, respectively.

Effects of gas concentrations of ethylene and carbon dioxide on seed germination

The effects of combinations of ethylene and CO₂ on seed germination using seeds stored under dry and moist conditions at 5°C are shown in Table 1. In a combined gas treatment of ethylene and CO₂, seed germination increased. However, there was a difference in effect of ethylene and CO₂ on germination depending on storage conditions. In seeds stored under a dry condition at 5°C, germination of over 60% was obtained by application of 0.7-35 ppm ethylene plus 4% CO₂. In seeds stored under other conditions, over 60%

Fig. 2. Effects of ethylene, CO₂ or their combination and two storage conditions on the germination of *Monochoria* seeds.

* The absorbents used were 0.25 M Hg (ClO₄)₂ for C₂H₄ and 20% KOH for CO₂.

To test germination: after 2 months in storage, the seeds were placed on wet filter paper in a 55 ml-glass tube which was sealed with a rubber stopper.

Each point is the mean of 3 experiments (±SE).

See <Experiment II> for detail of gas composition.

germination was obtained by 8-35 ppm ethylene plus 0.1-4% CO₂, or 0.3% CO₂ or by 0.7-35 ppm ethylene plus 1-4% CO₂ or 0-35 ppm ethylene plus 4% CO₂. In the presence of the absorbent of CO₂, the seeds were induced to germinate by ethylene. However, in the presence of the absorbent of ethylene, CO₂ had minimal effect on germination.

Discussion

The response of *Monochoria* seeds to ethylene or CO₂ depends upon their storage conditions.

About 2 months storage under a dry condition at 5°C seems to maintain a seed dormancy compared to the other storage conditions (i.e., a moist condition at 5°C or a field condition). Seeds showed a significant response only to high concentrations of both ethylene and CO₂. On the other hand, those stored under a moist condition, which seems to promote a loss of seed dormancy were significantly affected by low concentrations of ethylene and CO₂. The highest germination percentage of seeds stored under both conditions was

Table 1. Effect of ethylene and CO₂ on seed germination of *Monochoria*.

Storage of Seeds	Initial Concentration of C ₂ H ₄	KOH	Initial Concentration of CO ₂ %			
			0.1	0.3	1	4
Dry Condition at 5°C	ppm		Germination %			
	Hg(ClO ₄) ₂	1.5±0.5	0.0±0.0	0.5±0.5	0.5±0.5	1.0±0.0
	0.0	0.5±0.5	1.5±0.5	2.0±1.0	1.5±0.5	45.5±5.5
	0.7	1.5±0.5	3.5±0.5	4.0±1.0	9.0±1.5	63.0±4.0
	8	2.5±0.5	7.0±1.0	4.5±1.5	1.5±4.5	72.0±7.0
Moist Condition at 5°C	35	4.0±0.0	18.0±2.0	20.5±3.5	50.0±9.0	88.0±8.0
	Hg(ClO ₄) ₂	1.5±0.5	0.0±0.0	0.0±0.0	5.5±0.5	38.0±3.0
	0.0	4.5±0.5	6.5±1.5	31.0±2.0	48.5±6.5	72.0±4.0
	0.7	16.0±3.0	49.0±4.0	52.5±4.5	71.0±3.0	73.0±4.0
	8	48.0±3.0	68.0±4.0	61.5±4.5	63.5±3.5	70.0±5.0
	35	50.0±6.0	80.0±14.0	64.0±6.0	73.5±5.5	76.5±3.5

a) Mean of 2 experiments (±SE).

b) The absorbents used were 0.25 M Hg(ClO₄)₂ for C₂H₄ and 20% KOH for CO₂.

c) In all glass tubes, ambient air (21% O₂) was used.

d) To test germination: After 2 months in storage, the seeds were placed on wet filter paper in a glass tube sealed with a rubber stopper.

obtained in the combined presence of the two gases (ethylene and CO₂), while in the presence of absorbents of these gases, germination percentage declined significantly. In the presence of the absorbent of CO₂, seeds were induced to germinate by ethylene. However, the effect of CO₂ in the presence of the absorbent of ethylene on germination was slight. The results appear to indicate that the effect of ethylene on seed germination is more significant than that of CO₂.

The stimulus of seed germination by ethylene and the effect of CO₂ on ethylene production is well known^{3,4,6-8,10}). In fact, *Monochoria* seeds require little oxygen for germination. Under 60% oxygen, the seeds stored under any of the conditions exhibited a significantly decreased germination percentage, while under 0.2% oxygen germination was high whatever the condition of storage.

Yang and Hoffmann¹⁹) noted that the concentrations of CO₂ giving half maximal promotion of 1-aminocyclopropane-carboxylic acid (ACC)-dependent ethylene production are only several-

Fig. 3. Scanning electron micrograph of a longitudinal section of a *Monochoria* seed (×85).

The seed was coated with gold immediately after harvest.

Em, Embryo; Sc, Seed coat.

fold higher than the ambient level of CO₂ in the atmosphere, meaning that an important factor regulating the evolution of ethylene is CO₂ metabolism^{6,7,19}. Under the hypoxic conditions of a paddy field, germination of *Monochoria* seeds seems to be stimulated by the endogenous ethylene in the seeds, which is produced by exogenous CO₂ in the atmosphere.

As reference data, Fig. 3 shows an electron micrograph of a longitudinal section of a *Monochoria* seed. The embryo was fully developed morphologically when the seed was harvested. If the seed was placed under low oxygen conditions the radicle appeared within 2-3 days, meaning that *Monochoria* seeds require low oxygen for radicle protrusion. But when grown under a low oxygen (0.2%) concentration *Monochoria* seedlings were albino with abnormal roots^{1,9}.

Yamasue and Ueki¹⁸) reported that seeds of *Echinochloa oryzicola* under the anaerobic condition showed good growth of the coleoptile with no radicle protrusion. In an extract from the seeds incubated in air, alcohol dehydrogenase activity decreased although there was no increase of cytochrome c oxidase activity until the initiation of radicle growth. They therefore suggested that the radicle protrusion and growth were dependent on the aerobic respiration involving cytochrome c oxidase. Kordon^{11,12}) also found that over 90% of the rice seeds germinated in vacuo (O₂ deficit) within 7 days, although there was only coleoptile emergence with no greening or root emergence. Thus, the effect of oxygen on *Monochoria* seeds after germination may be related to biosynthesis of chlorophyll or normal growth of roots.

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C₂H₄ および CO₂ がコナギ (*Monochoria vaginalis* var. *plantaginea*) 種子の発芽におよぼす影響

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摘 要

コナギ (*Monochoria vaginalis* var. *plantaginea*) 種子の発芽と、とくに土壤中の気相として想定される C₂H₄, CO₂, O₂ とそれらを組み合わせたガス条件との関係を検討した。高発芽歩合は C₂H₄ と CO₂ の両方の存在によって認められ、C₂H₄ および CO₂ の両方の吸収剤が存在すると発芽歩合は著しく減少した (Table 1)。CO₂ 吸収剤の存在下で外生的に与えた C₂H₄ は発芽を増加させたが、一方 C₂H₄ 吸収剤

の存在下での外生的 CO₂ の発芽への影響は極めて小さかった。しかし、外生的 CO₂ 濃度の高い場合は発芽歩合は高まった (Table 1)。これらのことから、コナギ種子の発芽は CO₂ より C₂H₄ に影響されること、また、外生的に与えた CO₂ 濃度の刺激で生成される内生的 C₂H₄ により発芽が増加することが示唆された。

さらに、コナギ種子は高酸素 (60% O₂) 下では発芽歩合が著しく減少し、低酸素 (0.2% O₂) 下では高い発芽歩合を示した (Fig. 2)。事実、休眠覚醒した種子でさえ通気状態でろ紙を敷いたベトリ皿では発芽が認められなかった (Fig. 1)。これらのことは、コナギ種子の発芽にはほとんど酸素を必要としないことを証明している。

キーワード: エチレン, コナギ種子, 酸素, 二酸化炭素, 発芽。

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