J Plant Growth Regul (1988) 7:45–58

Journal of
Plant
Growth
Regulation

Effects of 2,5-Norbornadiene on Cocklebur Seed Germination and Rice Coleoptile Elongation in Response to CO_2 and C_2H_4

K. Ishizawa, M. Hoshina, K. Kawabe, and Y. Esashi

Department of Biological Science, Tohoku University, Kawauchi, Sendai 980. Japan.

Received August 10, 1987; accepted November 11, 1987

Abstract. To examine in more detail the mechanisms of cocklebur (*Xanthium pennsylvanicum* Wallr.) seed germination and rice (*Oryza sativa* L. cv. Sasanishiki) coleoptile elongation that were responsive to both C_2H_4 and CO_2, the effects of NBD (2,5-norbornadiene), a cyclic olefin known as a competitive inhibitor of C_2H_4, on those phenomena were tested under various conditions. NBD strongly inhibited germination of cocklebur seeds and their axial and cotyledonary growth. The NBD effects were significantly negated by endogenously evolved and exogenously applied CO_2 regardless of incubation temperature. Similarly, the inhibitory NBD effect was negated by C_2H_4 at 23°C, but at 33°C a low concentration (3 μl/L) of C_2H_4 rather enhanced the inhibitory NBD effect. This phenomenon reflected the growth responses of the tip zone of axial tissues in cocklebur seeds to NBD and C_2H_4, in which both gases were antagonistic in regulating the axial growth at 23°C but additive in inhibiting it at 33°C. Maximal negation of these inhibitory NBD effects was brought about by simultaneous application of CO_2 and C_2H_4. Similarly, elongation of rice coleoptiles was suppressed by NBD, and when they were immature, its inhibitory action was counteracted by both C_2H_4 and CO_2, especially during simultaneous application. However, the inhibitory NBD effect was completely negated by C_2H_4 applied alone at concentrations above 500 μl/L regardless of the physiological age of coleoptiles. These inhibitory NBD effects are additional evidence suggesting that C_2H_4 acts as a growth regulator in both cocklebur seed germination and rice coleoptile elongation. That NBD was capable of counteracting CO_2 action in some cases but was incapable of negating inhibitory C_2H_4 action, such as that observed in cocklebur seeds, suggests that NBD acts with some side effects besides being a competitive inhibitor of C_2H_4 actions.

NBD (2,5-norbornadiene), a cyclic olefin, was used for analysis of C_2H_4-involving phenomena in plants because it is a competitive inhibitor of C_2H_4 action (Sisler and Pian 1973, Sisler et al. 1983, 1985, Sisler and Yang 1984, Veen 1985, Hoekstra and Weges 1986, Curtis 1987). Kepczynski and Karssen (1985), Saini et al. (1986), and Abeles (1986) found that NBD inhibits seed germination and that its inhibitory effect is competitively counteracted by C_2H_4. From these results it was suggested that endogenous C_2H_4 plays an important role in seed germination.

CO_2 has been known to act as a competitive inhibitor of C_2H_4 action (Burg and Burg 1967). However, both CO_2 and C_2H_4 stimulated the germination of cocklebur seeds (Katoh and Esashi 1975, Esashi et al. 1978) and the elongation of rice coleoptiles (Ku et al. 1970, Ishizawa and Esashi 1984, Raskin and Kende 1983) in which CO_2 was additive to or synergistic with C_2H_4. These findings have raised doubts as to whether CO_2 is an actual competitive inhibitor of C_2H_4 or not. More recently, Satler and Kende (1985) found the full recovery from NBD inhibition of rice coleoptile elongation by C_2H_4. However, the effect of the ambient CO_2 levels on the experiments was not examined. In cocklebur seed germination, on the other hand, CO_2 was capable of counteracting C_2H_4 under some conditions, in which both gases were applied at temperatures above 27°C to seeds presoaked for more than 8 h at 23°C. The results obtained with cocklebur seeds suggest that the effects of NBD on cocklebur seed germination and rice coleoptile elongation may change depending on experimental conditions, and that NBD may have some bearing not only on C_2H_4 but also on CO_2 in regulating cocklebur seed germination and rice coleoptile elongation. If so, NBD may be useful to shed further light on the interrelation between CO_2 and C_2H_4 actions.

Materials and Methods

Germination Test

Most experiments were performed using the lower cocklebur (*Xanthium pennsylvanicum* Wallr.) seeds, which were harvested in the fall of 1985, after-ripened in dry storage for 5 months at room temperature, and stored at 8°C until used. Triplicate samples of 20 seeds, uniform in size, were placed on 2 disks of filter paper wetted with 4 ml of distilled water in a 125-ml flask. The flasks contained small glass tube(s) with 0.5 ml of 2.5 M NaOH as a CO_2 absorbent and/or with liquid NBD in microliter quantities and they were sealed with a rubber stopper. Then necessary volumes of CO_2 and C_2H_4 were syringed through the stopper into the flasks, and the needle perforations on the stoppers were covered with a piece of adhesive vinyl tape. The flasks were placed in the dark at 23°C. In an experiment at 33°C, however, after-ripened upper cocklebur seeds were used instead of the lower ones.

Axial and Cotyledonary Growth

Axial and cotyledonary tissues were separated 3 mm below the tip of the embryonic axis of lower cocklebur seeds according to procedures previously described (Esashi and Katoh 1975). Ten axial and 14 cotyledonary segments were presoaked for 5 h at room temperature, decoated, washed several times with tap water, rinsed with distilled water, blotted dry, weighed, and placed in the flasks as in the germination test. After specified periods of incubation at 23 or 33°C, their fresh weights were again measured. Results are the means ± SE of four replicates as percent increase of fresh weight.

Zonal Growth in Axial Tissues

Zonal growth of axial tissue of cocklebur seeds in response to NBD in combination with CO_2 and/or C_2H_4 was tested according to previously described methods (Esashi et al. 1987b). Prior to decoating, lower cocklebur seeds were presoaked for 12 h at 23°C. The axial 3.0-mm tissues, containing a radicle tip, an embryonic hypocotyl, and cotyledonary petioles of 17 seeds were marked with black ink for dividing into four zones at an interval of 0.75 mm from the axial tip and exposed to various gases in a 400-ml glass vessel for described periods. Growth of each axial zone was measured under magnification (13 ×) and shown by the means ± SE of 17 determinations.

Rice Coleoptile Elongation

Rice (*Oryza sativa* L. cv. Sasanishiki) caryopses were husked, sterilized in 5% sodium hypochloride solution for 20 min, and washed thoroughly with tap water. The husked caryopses were sown on a cotton net spread on a Petri dish filled with deionized water, germinated, and grown under saturated moisture at 30°C in the dark for described periods. The small Petri dishes with 17 rice seedlings, selected for uniformity, were put in a 400-ml glass vessel for gas treatments. After incubation at 30°C in the dark for described periods, the coleoptile lengths were measured, and their elongation was expressed as the difference between the initial and final lengths.

Results

Inhibition of Germination of Lower Cocklebur Seeds by NBD

Liquid NBD at amounts above 3 μl strongly inhibited germination of lower cocklebur seeds (Fig. 1). At a saturation level of 100 μl, NBD almost completely suppressed germination, suggesting that the germination of cocklebur seeds is controlled by endogenously evolved C_2H_4 if NBD is an actual competitive inhibitor of C_2H_4.

The germination-inhibiting effect of NBD was very pronounced when NBD was applied early in the soaking process, and it decreased gradually with progress of germination (Fig. 2). However, NBD was capable of inhibiting germination even when applied at 20 h, at which time the lower seeds started to germinate (Fig. 3).

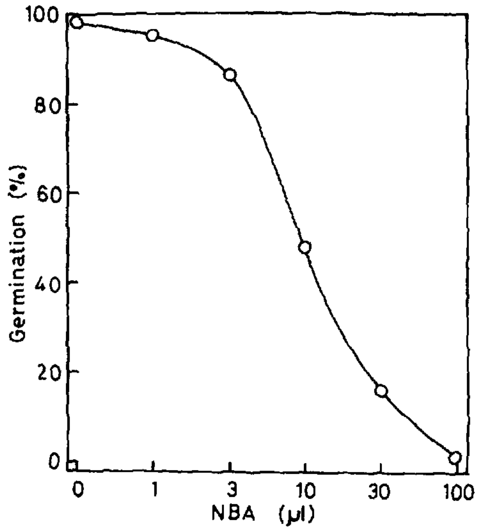

Fig. 1. Effect of NBD on the germination of lower cocklebur seeds at 23°C. Nonimbibed lower seeds were incubated at 23°C in the dark for 56 h in the presence of different amounts of liquid NBD in a 125-ml flask but in the absence of a CO_2 absorbent.

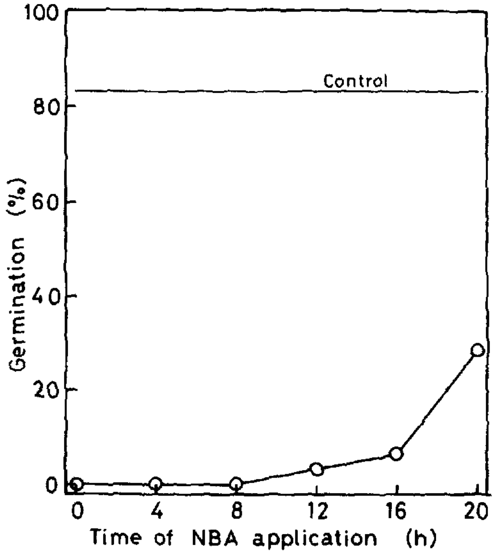

Fig. 2. Time-sequence responses in the germination at 23°C of lower cocklebur seeds to NBD. Nonimbibed lower seeds were exposed to 10 µl NBD at different times after the start of soaking at 23°C, except for the control. Data were taken 38 h after soaking in the absence of a CO_2 absorbent.

Removal of Germination-Inhibiting Effect of NBD by C_2H_4 and/or CO_2

The germination-inhibiting effect of NBD, applied during an early period of soaking, was almost completely negated by C_2H_4 and CO_2 in combination, although germination of NBD-pretreated seeds occurred very slowly in the C_2H_4- and CO_2-free air as compared with the nonpretreated seeds (Fig. 3). CO_2 was more effective than C_2H_4 in negating the inhibitory NBD effect, although it was less effective than C_2H_4 during the earlier periods of posttreatment. These results imply that NBD has some connection not only with C_2H_4 but also with CO_2 in regulating seed germination.

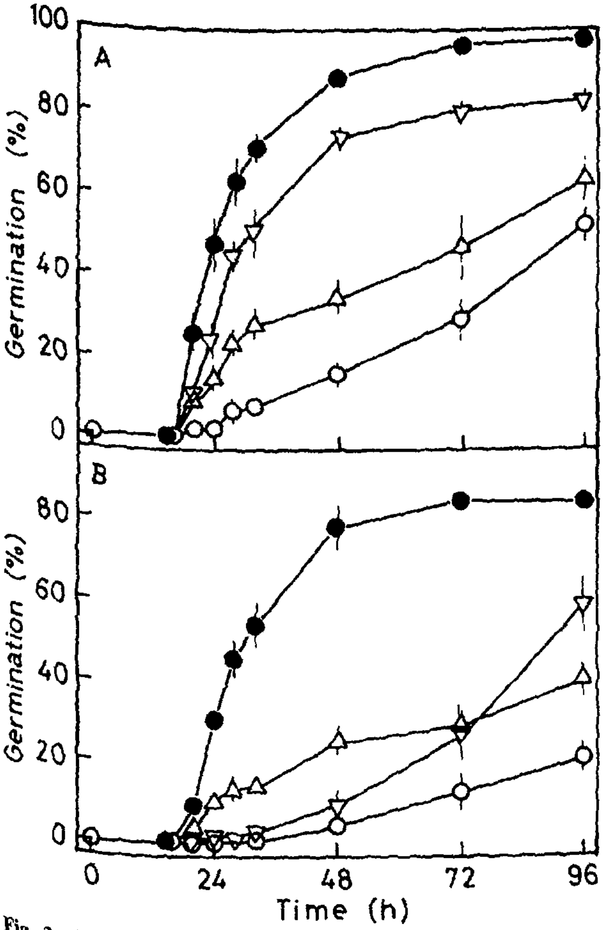

Fig. 3. Germination responses to C_2H_4 and/or CO_2 of NBD-pretreated lower cocklebur seeds at 23°C. Upon soaking, lower seeds were either exposed to CO_2- and C_2H_4-free air (A) or to air with 5 μl NBD (B) in a 125-ml flask for 16 h at 23°C, and then exposed to CO_2- and C_2H_4-free air (○), 3% CO_2 alone (▽), 10 μl/L C_2H_4 alone (△), or 3% CO_2 plus 10 μl/L C_2H_4 (●) at 23°C in the dark.

Interaction in the Regulation of Seed Germination Between NBD and CO_2 or C_2H_4

As suggested in Fig. 3, the germination-inhibiting effect of NBD was counteracted not only by C_2H_4 but also by CO_2 (Table 1). The NBD effect was greatly removed by C_2H_4 regardless of the presence of CO_2. On the other hand, 3% CO_2 could also alleviate NBD-induced inhibition. NBD inhibition was partially reduced even by the CO_2 that accumulated in flasks in the absence of NaOH. Moreover, the NBD-counteracting effects of both gases decreased with increasing the amounts of NBD. Thus, there was no apparent difference between C_2H_4 and CO_2 in counteracting NBD. It is thus doubtful that NBD is a specific inhibitor of C_2H_4 action.

Lower concentrations of C_2H_4 are known to prevent germination of pre-

Table 1. Interactions between NBD and C_2H_4 and/or CO_2 in the regulation of germination of lower cocklebur seeds at 23°C.

NBD (μl)	C_2H_4	Germination (%)		
		+NaOH	−NaOH	$+CO_2$
0	−	45.0 ± 3.4	71.7 ± 5.6	98.3 ± 1.4
	+	93.3 ± 2.6	100.0	100.0
3	−	18.3 ± 3.2	28.5 ± 4.8	35.0 ± 5.2
	+	48.3 ± 4.1	60.0 ± 3.5	61.6 ± 6.0
10	−	3.3 ± 1.2	6.7 ± 1.8	23.8 ± 3.9
	+	33.5 ± 4.0	40.0 ± 5.6	78.7 ± 4.4

Lower seeds were exposed to different amounts of liquid NBD in the absence or presence of 10 μl/L C_2H_4 with or without a CO_2 absorbent or 3% CO_2 from the start of water imbibition. Data after 48 h are shown as the means ± SE of triplicate samples.

Table 2. Interactions between NBD, C_2H_4, and CO_2 in the regulation of germination of presoaked upper cocklebur seeds at 33°C.

NBD (μl/125 ml)	C_2H_4 (μl/L)	Germination (%)	
		$-CO_2$	$+CO_2$
0	0	81.6 ± 3.3	95.0 ± 1.5
0	3	29.0 ± 5.2	91.9 ± 0.8
2	0	44.4 ± 6.0	65.1 ± 5.6
2	3	17.5 ± 2.0	59.4 ± 6.2
0	0	85.9 ± 2.4	86.0 ± 1.7
0	3	34.2 ± 2.9	91.7 ± 2.2
10	0	8.2 ± 1.0	38.0 ± 3.6
10	3	0	23.4 ± 4.5
0	0	80.3 ± 2.9	96.7 ± 1.2
0	30	53.4 ± 4.8	91.6 ± 3.2
10	0	15.0 ± 0	32.5 ± 3.8
10	30	29.2 ± 4.5	80.5 ± 4.9

Upper seeds presoaked at 23°C for 2 weeks were exposed to different amounts of liquid NBD in combination with C_2H_4 and/or 3% CO_2 for 40 h at 33°C. Data are the means ± SE of triplicate samples.

soaked cocklebur seeds at higher temperatures (Esashi et al. 1986). Therefore, if NBD is a specific inhibitor of C_2H_4 action, NBD would be expected to promote the germination of cocklebur seeds at 33°C. However, NBD at 2 or 10 μl failed to stimulate germination of upper cocklebur seeds at 33°C and strongly inhibited it (Table 2). Moreover, the inhibitory effect of NBD on germination at 33°C was not negated by C_2H_4. On the contrary, the inhibitory effect of C_2H_4 was further enhanced by NBD. In contrast, at 33°C, CO_2 counteracted not only C_2H_4 but also NBD, thus alleviating their germination-inhibiting effects. However, NBD inhibition was slightly counteracted by high C_2H_4 concentration of 30 μl/L (Table 2), which was less inhibitory than 3 μl/L. This phenomenon may be associated with the dual actions of C_2H_4 in regulating the growth

Table 3. Effects of NBD on the growth of axial tissues of cocklebur seeds at 23 or 33°C in combination with CO_2 and/or C_2H_4.

Temp (°C)	C_2H_4 (µl/L)	CO_2 (3%)	Fresh weight increase (%)		
			None	2 µl NBD	20 µl NBD
23	0	–	53.5 ± 2.0	30.3 ± 1.6	11.6 ± 1.6
	0	+	66.5 ± 2.6	50.4 ± 1.9	38.6 ± 1.4
	1	–	69.4 ± 4.8	41.7 ± 2.7	30.8 ± 3.4
	1	+	70.7 ± 0.4	58.8 ± 3.8	48.1 ± 2.6
	10	–	71.7 ± 4.6	60.1 ± 4.3	43.2 ± 2.4
	10	+	76.4 ± 7.9	61.8 ± 4.8	50.9 ± 3.1
33	0	–	102.6 ± 4.4	99.1 ± 6.2	61.6 ± 7.3
	0	+	114.0 ± 3.7	104.4 ± 4.1	70.5 ± 2.1
	1	–	111.4 ± 6.9	96.8 ± 7.0	65.9 ± 7.4
	1	+	112.6 ± 3.1	107.5 ± 5.3	72.0 ± 3.9
	10	–	118.1 ± 5.3	99.3 ± 6.3	68.4 ± 4.1
	10	+	120.6 ± 3.0	109.4 ± 3.8	74.8 ± 6.9

Axial segments from lower cocklebur seeds were presoaked for 5 h at room temperature and exposed in 125-ml flasks to variously combined gases for 17 h at 23 or 33°C. Data are the means ± SE of four replicate samples.

Table 4. Effects of NBD on the growth of cotyledonary tissues of cocklebur seeds at 23 or 33°C in combination with CO_2 and/or C_2H_4.

Temp (°C)	C_2H_4 (µl/L)	CO_2 (3%)	Fresh-weight increase (%)		
			None	2 µl NBD	20 µl NBD
23	0	–	55.1 ± 3.4	8.4 ± 0.7	1.9 ± 0.4
	0	+	64.1 ± 1.2	24.5 ± 3.0	9.7 ± 1.6
	1	–	68.4 ± 1.9	31.8 ± 4.2	16.1 ± 1.9
	1	+	69.0 ± 1.0	41.4 ± 2.5	26.5 ± 4.0
	10	–	66.9 ± 2.6	44.7 ± 3.1	25.0 ± 1.4
	10	+	64.5 ± 2.1	57.6 ± 1.8	27.6 ± 1.1
33	0	–	55.8 ± 3.2	28.9 ± 1.1	17.6 ± 1.6
	0	+	66.5 ± 2.6	50.4 ± 1.9	38.6 ± 1.4
	1	–	69.4 ± 4.8	41.7 ± 2.7	30.8 ± 3.4
	1	+	70.7 ± 0.4	58.8 ± 3.8	48.1 ± 2.6
	10	–	71.7 ± 4.6	60.1 ± 4.3	43.2 ± 2.4
	10	+	71.9 ± 6.4	61.8 ± 4.8	50.9 ± 3.1

Cotyledonary segments from lower cocklebur seeds were presoaked for 5 h at room temperature and exposed in 125-ml flasks to variously combined gases for 64 h at 23°C and for 46 h at 33°C, respectively. Data are the means ± SE of four replicate samples.

of cocklebur seed axial tissues—i.e., inhibition at their tip zone and promotion at their distal zone (Esashi et al. 1987b).

Inhibition by NBD of the Axial and Cotyledonary Growth of Cocklebur Seeds and Effect of CO_2 and C_2H_4

NBD suppressed both axial (Table 3) and cotyledonary (Table 4) growth in cocklebur seeds at 23 and 33°C. The degree of growth inhibition by NBD was less at 33°C than at 23°C. Importantly, CO_2 as well as C_2H_4 counteracted the NBD effect at 23°C. The similar interrelations between NBD and CO_2 or C_2H_4 were found even at 33°C in the cotyledonary growth (Table 4). In all cases, maximum recovery from the inhibitory NBD effect was obtained by exposure to both CO_2 and C_2H_4 in combination. However, the additive inhibition by NBD and C_2H_4 at its lower concentration as seen in seed germination at 33°C (Table 2) was not found in the growth of either tissue at 33°C.

Zone Growth of Axial Tissues in Response to NBD and Its Mediation by CO_2 and/or C_2H_4

Zones A and B, including the radicle tip, and zones C and D, corresponding to the embryonic hypocotyl and cotyledon petioles, respectively, have been known to respond differently to C_2H_4 depending on temperature: growth of zones A and B was stimulated by C_2H_4 at 23°C but inhibited at 33°C, whereas C_2H_4 stimulated the growth of zones C and D regardless of temperature (Esashi et al. 1987b). The effects of NBD on the zonal axial growth at 23 or 33°C were examined in the absence or presence of CO_2 and/or C_2H_4 (Figs. 4, 5). NBD strongly inhibited growth of all axial zones regardless of temperature, but zone A was less responsive to NBD at 33°C than at 23°C (compare Figs. 4 and 5). At 23°C, C_2H_4, which is known to greatly promote the axial growth in zones C and D (Esashi et al. 1987b), markedly negated the growth inhibition by NBD in zones C and D, whereas CO_2, which promotes growth in zones A and B, negated the NBD effect in zones A and B. Simultaneous application of CO_2 and C_2H_4 resulted in maximal recovery in zone B.

At 33°C, on the other hand, CO_2 negated NBD inhibition of axial growth in all zones, but negation by C_2H_4 occurred only in zones C and D. The growth inhibition in zone A by NBD was rather increased by C_2H_4 (Fig. 5). That is, C_2H_4, unlike CO_2, could not counteract NBD in zone A. Interestingly, growth in the presence of CO_2 was greater than that in air, which implied that CO_2 counteracted the growth inhibition at zone A by endogenously evolved C_2H_4.

Interaction in the Regulation of Rice Coleoptile Elongation Between NBD, CO_2, and C_2H_4

In rice coleoptiles as well as cocklebur seeds, NBD strongly inhibited the elongation. Its inhibitory action was partially removed by CO_2, and maximum negation occurred by simultaneous application of C_2H_4 and CO_2 (Table 5). C_2H_4 could always antagonize NBD, but CO_2 applied singly was effective only when the coleoptiles were immature at the time of application. Relatively mature coleoptiles were insensitive to CO_2 but were sensitive to CO_2 in the presence of C_2H_4, as seen in the germination of secondarily dormant cocklebur seeds (Esashi et al. 1987). In these coleoptiles, CO_2 had little effect on NBD activity (Table 5).

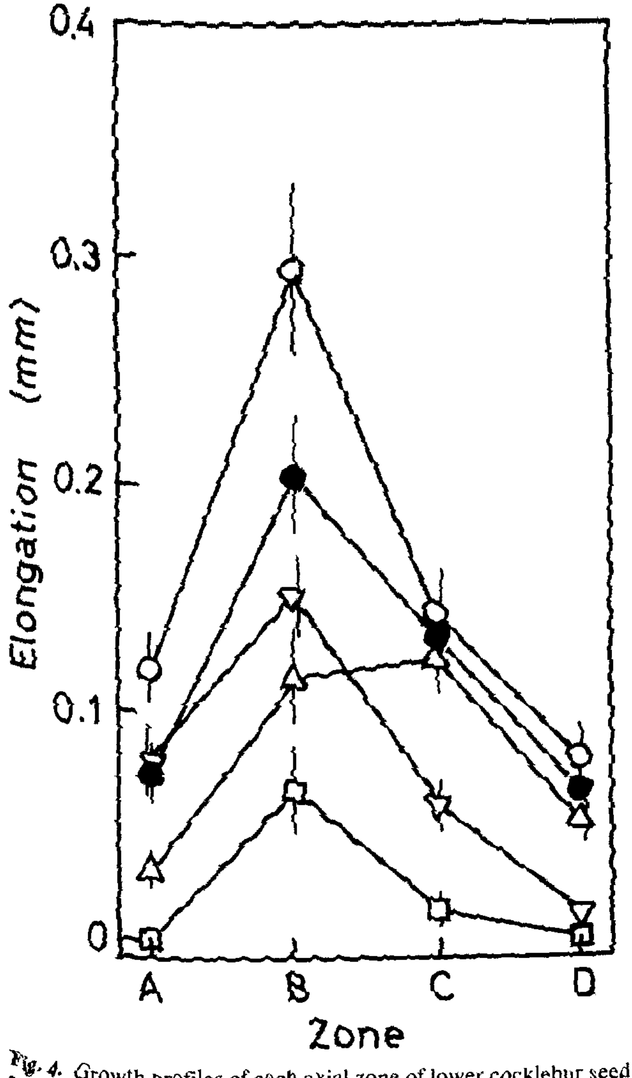

Fig. 4. Growth profiles of each axial zone of lower cocklebur seeds in response to various gases at 23°C. Seeds presoaked for 12 h at 23°C were decoated, marked for their axial tissues, and exposed in 400-ml glass vessels to CO_2-free air (○), 10 μl NBD with a CO_2 absorbent (□), 10 μl NBD plus 3 μl/L C_2H_4 with a CO_2 absorbent (△), 10 μl NBD plus 3% CO_2 (▽), and 10 μl NBD plus 3 μl/L C_2H_4 plus 3% CO_2 (●). Data were taken after 16 h of gas exposures and are shown by the means ± SE of 17 determinations.

A Complete Recovery by C_2H_4 Alone from NBD Inhibition

In Tables 1, 3, 4, and 5, negation of the inhibitory effects of NBD was maximum following simultaneous application of CO_2 and C_2H_4, but the degree of negation was incomplete in any case. If NBD is a specific competitive inhibitor of C_2H_4 action, then NBD inhibition should be completely negated by increasing concentration of C_2H_4. However, the inhibition by NBD of both the

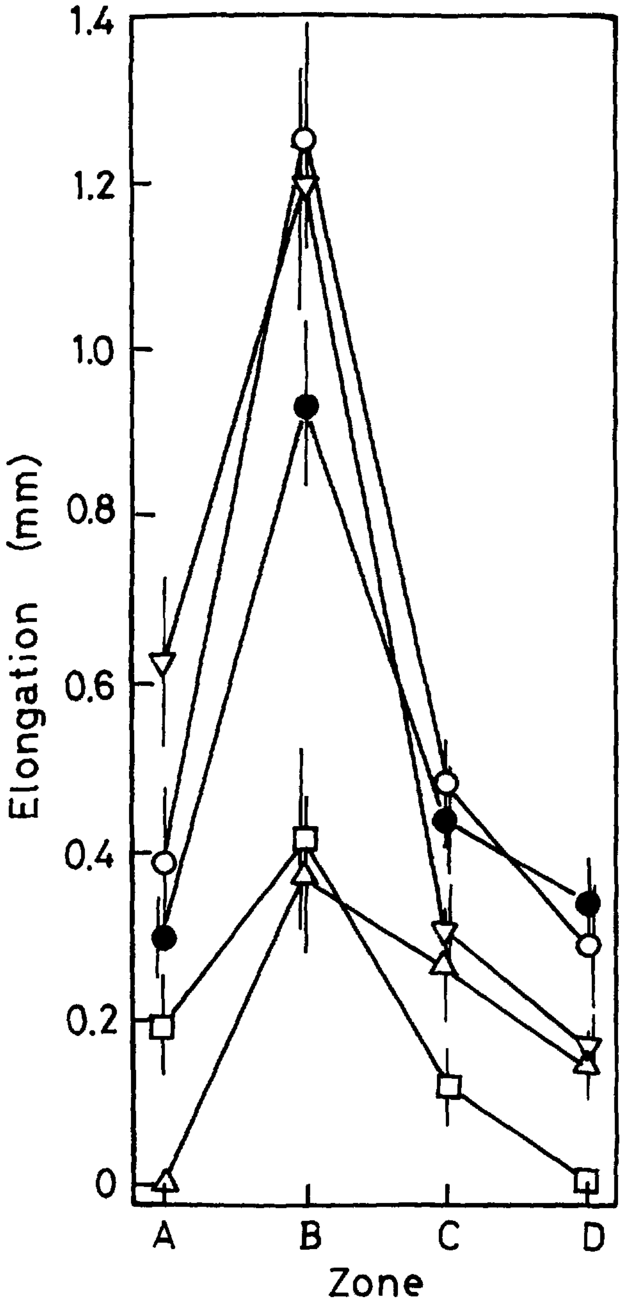

Fig. 5. Growth profiles of each axial zone of lower cocklebur seeds in response to various gases at 33°C. Treatments except for incubation temperature and symbols are the same as in Fig. 4. Data were taken after 14 h.

Table 5. Effects of NBD on the elongation of rice coleoptiles in combination with CO_2 and/or C_2H_4.

Exp	C_2H_4	CO_2	Growth increase (mm)		
			None	2 μl NBD	20 μl NBD
A	−	−	13.6 ± 0.5	9.5 ± 0.4	8.5 ± 0.4
	−	+	14.3 ± 0.4	10.5 ± 0.3	9.3 ± 0.3
	+	−	17.3 ± 0.4	15.1 ± 0.5	12.1 ± 0.4
	+	+	23.9 ± 0.7	16.5 ± 0.5	12.8 ± 0.5
B	−	−	6.1 ± 0.5	1.7 ± 0.4	0.9 ± 0.5
	−	+	5.8 ± 0.7	2.1 ± 0.4	1.4 ± 0.4
	+	−	11.6 ± 0.6	6.8 ± 0.5	3.9 ± 0.4
	+	+	15.3 ± 0.7	7.9 ± 0.5	5.4 ± 0.4

Rice seedlings growth for 36 h (A) or 62 h (B) were exposed to different amounts of liquid NBD in 400-ml glass vessels at 30°C in the absence or presence of 5 μl/L C_2H_4 and/or 3% CO_2. Data were taken after 30 h in A and after 24 h in B and are shown by means ± SE of 15–18 determinations. Initial coleoptile lengths were 2.6 ± 0.1 mm in A and 11.5 ± 0.2 mm in B.

Table 6. Recovery of elongation of rice coleoptiles from its NBD inhibition by C_2H_4.

Exp	C_2H_4 (μl/L)	Growth increase (mm)	
		Control	20 μl NBD
A	0	21.1 ± 0.5	15.2 ± 0.4
	10	23.5 ± 0.6	18.5 ± 0.2
	100	27.0 ± 0.6	24.7 ± 0.7
	500	25.6 ± 0.8	25.3 ± 1.2
	1000	24.8 ± 0.7	23.5 ± 0.9
	5000	24.9 ± 0.7	23.5 ± 0.8
B	0	1.7 ± 1.0	0.7 ± 0.6
	50	7.8 ± 0.4	5.4 ± 0.8
	100	7.6 ± 0.5	6.4 ± 0.5
	500	7.6 ± 0.3	8.5 ± 0.4

Rice seedlings grown for 30 h (A) or 72 h (B) were incubated with or without 20 μl NBD and exposed to different concentrations of C_2H_4 for 42 h or 24 h, respectively, at 30°C in the presence of a CO_2 absorbent in the dark. Data are shown by means ± SE of four replicate experiments. Initial lengths of coleoptiles were 0.8 ± 0.1 mm in A and 13.5 ± 0.3 mm in B.

germination of cocklebur seeds and the growth of cocklebur cotyledons was not completely negated by C_2H_4, even if its concentration was increased up to 10,000 μl/L (data not shown). In rice coleoptiles, on the contrary, increase in C_2H_4 concentration induced progressively higher recovery from the NBA inhibition of their elongation even in the absence of CO_2, and C_2H_4 at 500 μl/L or more completely negated the NBD inhibition, regardless of the physiological age of coleoptiles (Table 6).

Discussion

If NBD is an actual competitive inhibitor of only C_2H_4 action, as has been presumed by Sisler and Pian (1973), the findings that NBD inhibits germination of cocklebur seeds (Figs. 1, 2; Table 1), and growth of their axial and cotyledonary tissues (Tables 3, 4) and elongation of rice coleoptiles (Tables 5, 6) may be additional evidence for confirming that all of these phenomena are mediated by endogenously evolved C_2H_4. However, evidence showing that NBD is a specific antagonist against only C_2H_4 was not obtained. The inhibitory effects of NBD on seed germination, axial and cotyledonary growth, and coleoptile elongation were alleviated not only by C_2H_4 but also by CO_2 (Figs. 3, 4; Tables 1, 3, 4, 5). The inhibition by NBD of cocklebur seed germination at 33°C was increased by C_2H_4 at a low concentration (Table 2), and the NBD inhibition of axial tip zones in cocklebur seeds was also strengthened by C_2H_4 (Fig. 5). From these results, it is difficult to explain the action of NBD as a specific competitive inhibitor of C_2H_4 action.

We questioned the views of Burg and Burg (1967) that CO_2 is a competitive inhibitor of C_2H_4, because CO_2 and C_2H_4 are synergistic or additive in regulating cocklebur seed germination (Katoh and Esashi 1975) and its axial and cotyledonary growth (Esashi and Katoh 1975). Later, we reported that CO_2 and C_2H_4 act to regulate the elongation of cocklebur and bean hypocotyls (Gotoh and Esashi 1974a,b) and the germination of cocklebur seeds (Esashi et al. 1987) in different tissue zones. In the former cases, the gases are antagonistic, but in the latter case they behave synergistically. Recently, we found that under some conditions the interaction between CO_2 and C_2H_4 shifts from synergism toward antagonism, and came to the view that CO_2 and C_2H_4 are antagonistic when the ratio of alternative respiration to cytochrome respiration is high, whereas they are synergistic when low (Esashi et al. 1986, 1987a,c). In these cases, there was a prerequisite that the plant tissues favor the cytochrome path rather than the alternative path for growth. Similarly, NBD was antagonistic to C_2H_4 when CO_2 and C_2H_4 were synergistic (Tables 1, 3, 4, 5), and it was additive to C_2H_4 when CO_2 and C_2H_4 were antagonistic (Table 2, Fig. 5). These phenomena seem to be associated with the reversal of C_2H_4 action on seed germination depending on temperature shift. On the other hand, the interrelation between CO_2 and NBD did not change in all cases: NBD effects were always negated by CO_2 (Fig. 4; Tables 1, 3, 4), suggesting that NBD can counteract CO_2 actions with some side effect besides being a competitive inhibitor of C_2H_4 action.

In rice coleoptiles, the growth-inhibiting effect of NBD was partially negated not only by C_2H_4 but also by CO_2, as in cocklebur, when they were immature, but it was mitigated only by C_2H_4 when more mature (Table 5). However, the inhibitory NBD effect was almost completely removed in the presence of C_2H_4 at high concentrations above 500 μl/L even without CO_2 (Table 6), which agreed with the finding by Satler and Kende (1985). Moreover, full recovery took place regardless of the physiological age of coleoptiles (Table 6). This result clearly shows that NBD is a specific competitive inhibitor of C_2H_4 action. This paradox from different experimental materials might be associated with differences in the involvement of CO_2. Probably, NBD acts as a typical competitive inhibitor against C_2H_4 only when the experimental systems are not greatly responsive to CO_2 applied alone.

In cocklebur, high concentration (30 μl/L) of C_2H_4 slightly counteracted the inhibitory NBD effect on germination at 33°C (Table 2), although even C_2H_4 at 10,000 µl/L could not completely negate the NBD-inhibited germination or cotyledonary growth (data not shown). On the other hand, C_2H_4 was inhibitory to radicle tip growth of axial tissue at 33°C (Fig. 5). Therefore, the NBD-counteracting action of higher concentraion of C_2H_4 seems to result from a superiority of C_2H_4-stimulated growth at subapical zones over C_2H_4-suppressed growth at the radicle tip zones.

References

Abeles FB (1986) Role of ethylene in *Lactuca sativa* cv 'Grand Rapids' seed germination. Plant Physiol 81:780–787

Burg SP, Burg EA (1967) Molecular requirements for the biological activity of ethylene. Plant Physiol 42:144–152

Curtis RW (1987) Antiethylene properties of $AgNO_3$ and 2,5-norbornadiene in light and dark in *Vigna radiata*. J Plant Growth Regul 6:41–56

Esashi Y, Katoh H (1975) Dormancy and impotency of cocklebur seeds. III. CO_2- and C_2H_4-dependent growth of the embryonic axis and cotyledon segments. Plant Cell Physiol 16:707–718

Esashi Y, Okazaki N, Yanai N, Hishinuma K (1978) Control of the germination of secondary dormant cocklebur seeds by various germination stimulants. Plant Cell Physiol 19:1497–1506

Esashi Y, Saijoh Y, Ishida S, Oota H, Ishizawa K (1986) Reversal of ethylene action on cocklebur seed germiantion in relation to duration of pre-treatment soaking and temperature. Plant Cell Environ 9:121–126

Esashi Y, Fuwa N, Kurota A, Oota H, Abe M, (1987a) Interrelation between ethylene and carbon dioxide in relation to respiration and adenylate content in the pre-germination period of cocklebur seeds. Plant Cell Physiol 28:141–150

Esashi Y, Hase S, Kojima K, Fuwa N (1987b) Differential responsiveness in zonal growth of cocklebur seed axes to CO_2, C_2H_4, O_2 and respiration inhibitors. Plant Cell Physiol 28:163–169

Esashi Y, Kurota A, Abe M, Fuwa N (1987c) Further study on the involvement of cyanide-resistant respiration in the germination of cocklebur seeds. Plant Cell Physiol 28:151–161

Esashi Y, Kawabe K, Isuzugawa K, Ishizawa K (1987) Interrelation between carbon dioxide and ethylene on the stimulation of cocklebur seed germination. Plant Physiol (in press)

Gotoh N, Esashi Y (1974a) Stimulation by ethylene of axis and hypocotyl growth in bean and cocklebur seedlings. Physiol Plant 31:204–210

Gotoh N, Esashi Y, (1974b) Differential hormone responsibility in different growing zones of a bean hypocotyl. Planta 116:225–241

Hoekstra FA, Weges R (1986) Lack of control by early pistillate ethylene of the accelerated wilting of *Petunia* hybrida flowers. Plant Physiol 80:403–408

Ishizawa K, Esashi Y (1984) Gaseous factors involved in the enhanced elongation of rice coleoptiles under water. Plant Cell Environ 7:239–245

Katoh H, Esashi Y (1975) Dormancy and impotency of cocklebur seeds. I. CO_2, C_2H_4, O_2 and high temperature. Plant Cell Physiol 16:687–696

Kepczynski J, Karssen CM (1985) Requirement for the action of endogenous ethylene during germination of non-dormant seeds of *Amaranthus caudatus*. Physiol Plant 63:49–52

Ku HS, Suge H, Rappaport L, Pratt HK (1970) Stimulation of rice coleoptile growth by ethylene. Planta 90:333–339

Raskin I, Kende H (1983) Regulation of growth in rice seedlings. J Plant Growth Regul 2:193–203

Saini HS, Consolacion ED, Bassi PK, Spencer MS (1986) Requirement for ethylene synthesis and action during relief of thermoinduction of lettuce seed germination by combinations of gibberellic acid, kinetin, and carbon dioxide. Plant Physiol 81:950–953

Satler SO, Kende H (1985) Ethylene and the growth of rice seedlings. Plant Physiol 79:194–198

Sisler EC, Pian A (1973) Effect of ethylene and cyclic olefins on tobacco leaves. Tobacco Sci 17:68–72

Sisler EC, Reid MS, Fujino DW (1983) Investigation on the mode of action of ethylene in carnation senescence. Acta Hort 141:229–234

Sisler EC, Yang SF (1984) Ethylene, the gaseous plant hormone. BioScience 34:234–238

Sisler EC, Goren R, Huberman M (1985) Effect of 2,5-norbornadiene on abscission ethylene production in *Citrus* leaf explants. Physiol Plant 63:114–120

Veen H (1985) Antagonistic effect of silver thiosulphate or 2,5-norbornadiene on l-aminocyclopropane-l-carboxylic acid–stimulated growth of pistils in carnation buds. Physiol Plant 65:2–8