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Dormancy and impotency of cocklebur seeds III. CO_2- and C_2H_4-dependent growth of the embryonic axis and cotyledon segments

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Growth of segments of embryonic axes and cotyledons excised from dormant or nondormant cocklebur (*Xanthium pennsylvanicum* Wallr.) seeds and CO_2 and C_2H_4 production in these segments were examined in relation to the effects of temperature, CO_2 and C_2H_4. Both the nondormant axes and cotyledons grew even at low temperatures below 23°C, but the dormant ones failed to grow. There was only little difference in the CO_2 evolution between the nondormant and dormant ones, but both the axis and cotyledon segments from the dormant seeds exhibited little or no C_2H_4 productivity, unlike the nondormant ones, at low temperatures. However, a high temperature of 33°C caused rapid extension growth and C_2H_4 production even in dormant axes and cotyledons.

The inability of dormant axes and cotyledons to grow disappeared completely in the presence of C_2H_4 at fairly low concentrations. Removal of endogenous CO_2 and C_2H_4 reduced the growth in both axes and cotyledons, while exogenous CO_2 mainly enhaced axial growth although exogenous C_2H_4 strongly stimulated the growth of both organs. Regardless of the dormant status, however, maximum growth of these organs occurred when C_2H_4 was given together with CO_2. We suggest that dormancy in cocklebur seeds is due to the lack of growing ability in both organs, caused by the lack of C_2H_4 productivity in both dormant axes and cotyledons, particularly in the former.

By actually measuring the thrust force resulting from a germinating whole cocklebur seed and that from an excised growing embryonic axis of the seed under both anaerobiosis and aerobiosis, Esashi and Leopold (*8*) demonstrated that rupture of the seed coat on seed germination occurs when restraint imposed by the seed coat was overcome by the thrust developed by both the embryonic axis and cotyledons which were growing passively or actively. Based on a significant difference in the measured total thrust between the small, upper and large, lower cocklebur seeds, we documented that the inability of the after-ripened small seeds to germinate at room temperatures is not due to their dormant status, but to their impotency of not being able to develop enough total thrust to overcome the mechanical stress of the seed coat (*8*).

A number of papers (*4, 5, 7, 18, 23, 30, 34*) have reported that seed dormancy disappears when the seed coat or testa was punctured or removed, whereas, many others (*1, 26, 27, 31*) have stated that the embryonic axes of some plants grow when

excised from their nondormant seed, but fail to grow when excised from their dormant ones. Thus seed dormancy has roughly been divided into two categories: embryo-dormancy and coat-imposed dormancy (*33*). The after-ripened small and the freshly harvested cocklebur seeds may be said to be under coat-imposed and embryo dormancy, respectively, since the naked embryonic axes from the former grew but those from the latter did not (*32*).

On the other hand, both dormant and nondormant but impotent small cocklebur seeds have been known to be able to germinate under incubation at elevated temperatures (*15*, *28*). We have also found that exogenously applied C_2H_4, particularly in combination with CO_2, not only breaks the dormancy but also reduces the impotency of the after-ripened small seeds (*16*). Moreover, the C_2H_4 production of dormant seeds was far less than that of nondormant ones, and that of impotent small seeds was only half that of potent large ones (*15*), indicating that germination failure in both dormant and impotent seeds may be due to repressed and inferior C_2H_4 production, respectively. However, CO_2 was found to act differently with C_2H_4 in seed germination (*10*). We studied the growth responses of axis and cotyledon segments excised from dormant and nondormant large cocklebur seeds to exogenous C_2H_4 and CO_2, as well as the C_2H_4 and CO_2 production from these organs, at different temperatures in light to learn more about the mechanisms of seed dormancy and germination.

Materials and methods

Lower, large seeds of cocklebur (*Xanthium pennsylvanicum* Wallr.), selected for uniformity, were used throughout the experiments. They were excised at 3 mm below the tip of the embryonic axis for separation from the cotyledonary part, which was divided in half prior to water imbibition. The resultant axial and cotyledonary segments were each detached from the fragment of seed coat after imbibition of 4 hr for the axes and 6 hr for the cotyledons, washed several times with tap water, rinsed twice with distilled water, and then blotted before their initial fresh weights were measured. The degrees of their extension growth were expressed as percent increase in fresh weight, and all the results were averages of 3 replications of 12 segments per lot, represented by a typical one of at least 2 to 4 separate experiments.

For growth assay alone (Fig. 1, 8 and 9), the segments were placed on two layers of filter paper wetted with 2.5 ml distilled water in a 100-ml Erlenmeyer flask, to which one or two side vessels containing 1.5 ml 2.5 N NaOH, 0.25 M $Hg(ClO_4)_2$ or both were attached to trap endogenously evolved CO_2 and/or C_2H_4. Necessary volumes of CO_2 and C_2H_2 to give their indicated concentrations in the flasks were syringed through a rubber stopper fitted on the flask or side vessel, but in the experiments on CO_2 effect, an excess of CO_2 was added as needed to maintain the same O_2 tension in each flask as that in air. For measurement of evolved CO_2 and C_2H_4, the segments were arranged on a sheet (4×4 cm) of filter paper moistened with 1.0 ml distilled water and lined in a 22-ml vial, to which one or two side vessels containing the CO_2 and/or C_2H_2 absorbents were fitted. Gas samples were analyzed using an activated alumina column on a Hitachi gas chromatograph (Model 063–5050) equipped with a flame ionization detector for C_2H_4 and activated charcoal column on a Gaschro-Kogyo gas chromatograph (Model KOR-70) with

a thermal conductivity detector for CO_2. All manipulations were carried out under dim room light except for incubation period in the darkened thermostats.

Results and discussion

Segment growth: Growth of preimbibed axis and cotyledon segments incubated at 13°, 23° and 33°C is shown in Fig. 1. Just as the breaking of dormancy in cocklebur seeds and the potentiation of germination in the nondormant small seeds are aided by high temperatures (*15*, *28*), segments of both the axes and cotyledons responded readily to the increase of temperature with increased extension growth, regardless of the status of dormancy. Data in Fig. 1 further indicate the following facts:

First, at a temperature of 23°C, although the embryonic axes excised from the nondormant seed grew, the axes from the dormant seeds failed to, and the results agreed with those obtained by Wareing and Foda (*32*). Second, if isolated from dormant seeds, not only the excised axes but the excised cotyledons also failed to grow at 23°C while the cotyledons of the nondormant seeds could. These results clearly indicate the cause of seed dormancy in this plant to be in the inability of both the axis and cotyledons to grow at normal temperatures.

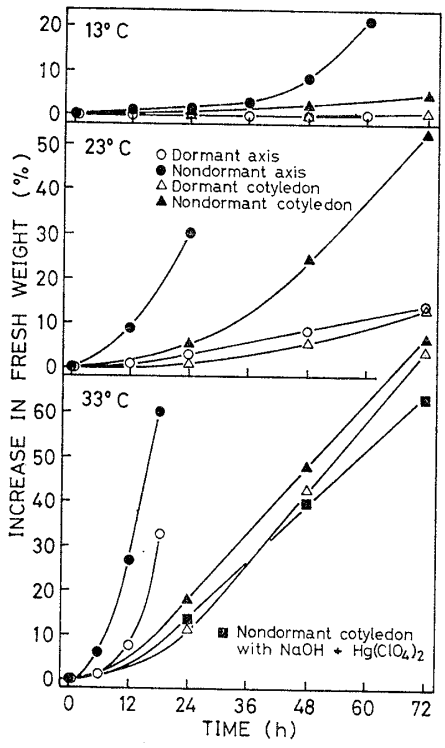

Fig. 1.

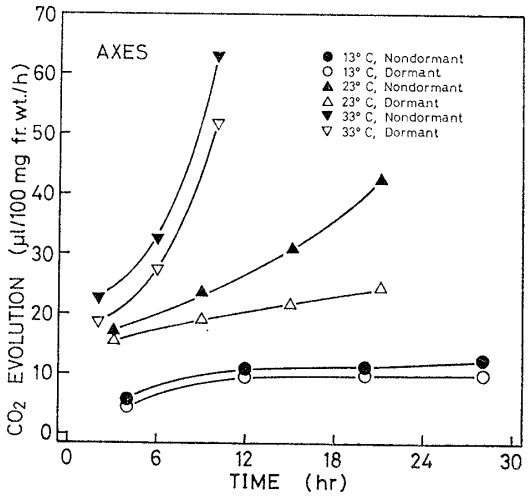

Fig. 2.

Fig. 1. *Extension growth in response to temperature of the embryonic axis and cotyledon segments excised from dormant and nondormant cocklebur seeds.* In one lot at 33°C, flasks were fitted with side vessels containing both NaOH and $Hg(ClO_4)_2$ solutions.

Fig. 2. *CO_2 evolution in dormant and nondormant axial segments in response to temperature.* The amounts of endogenously evolved CO_2 were measured at the time intervals of 4, 6 and 8 hr for 33°, 23° and 13°C, and the data were shown as the mean rate of CO_2 evolution at the mid points of these intervals.

Table 1 *CO_2 evolution of dormant and nondormant cotyledonary segments in response to temperature*

Temperature (°C)	Incubation time (hr)	CO_2 evolution (μl/100 mg fr. wt./hr)	
		Dormant	Nondormant
13	0–48	2.65	3.02
23	0–24	9.41	9.34
	24–48	15.15	19.10
33	0–24	13.55	17.75
	24–48	19.04	22.10

Data are given as the mean CO_2 evolution rate in each indicated period.

Third, as indicated in the previous paper (*8*), growth of embryonic axes occurred earlier than that of the cotyledons at all tested temperatures, suggesting that generally the axis begins to act earlier on rupture of the seed coat than a pair of cotyledons does. Very rapid axial growth at high temperature seems to favor an explanation for a normality of germination behavior under higher temperatures, at which the seed coat is broken only at the axial end of a seed (*10*).

Last, the faster extension growth of the nondormant cotyledons at 33°C was slightly reduced when endogenously evolving CO_2 and C_2H_4 were both trapped by NaOH and $Hg(ClO_4)_2$ solutions from the ambient atmosphere within the flasks.

Evolution of CO_2: CO_2 evolution of the axes and cotyledons of a different dormant status were examined, and Fig. 2 gave the results for the isolated axes while Table 1 that for the cotyledonary segments. In both organs, CO_2 evolution during the initial period of incubation rose with temperature increase regardless of the dormant state, and differences between the nondormant and dormant ones were not significant. At the low temperature of 13°C, both organs did not increase their CO_2 production during the incubation period. At 33°C, however, the CO_2 production increased strikingly, particularly in the axes, with incubation time regardless of the dormant status. In contrast, at 23°C, the CO_2 production increased only in the nondormant ones. These results suggest that there might be an ordinary respiratory process common to both seeds by which CO_2 was emitted, and that at 23°C the nondormant seeds could either exchange this process for another respiratory pathway which was closely associated with the commencement of axis and cotyledon enlargements or add another one to this, while the dormant seeds remained in a dormant state because of the failure to make such changes in their respiratory system. Since the presence of O_2 during the initial phase of the pregermination period is not indispensable for cocklebur seeds to germinate (*16*), the ordinary respiratory process may proceed depending weakly on O_2 or in anaerobiosis, as demonstrated by De la Fuente and Nicolás (*6*) with *Cicer arietinum*.

Evolution of C_2H_4: C_2H_4 evolution in excised embryonic axes was hardly detected during the initial period of incubation even at the higher temperature of 33°C (Fig. 3). Thereafter, the C_2H_4 productivity in the nondormant axes rapidly increased with time and reached maximum after 13 to 14 hr, followed by a subsequent decline. C_2H_4 production from the dormant axes began to rise with a lag period of about 10 hr, which coincides with the delay of their growth at 33°C in Fig. 1. Lowering the incubation temperature of the nondormant axes caused a decrease of the C_2H_4

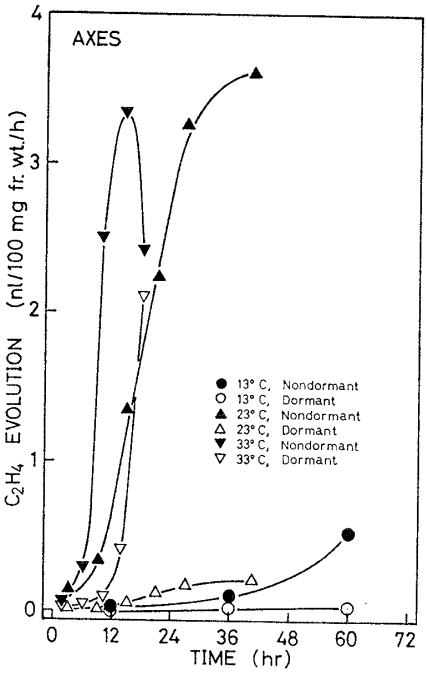

Fig. 3.

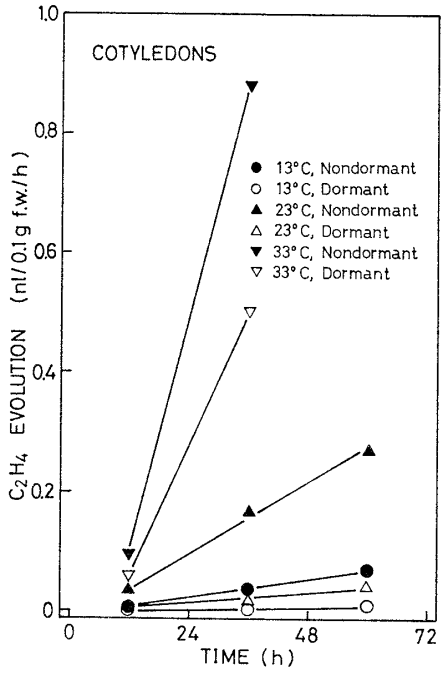

Fig. 4.

Fig. 3. *C_2H_4 evolution in dormant and nondormant axial segments in response to temperature.* Except for a 24-hr interval at 13°C, the other details are as given in Fig. 2.

Fig. 4. *C_2H_4 evolution in dormant and nondormant cotyledonary segments in response to temperature.* The amounts of endogenously evolved C_2H_4 were measured every 24 hr at various temperatures. Other details are as given in Fig. 2.

production with an increase in the lag period. More important, however, no dormant axial segments, unlike the nondormant ones, exhibited any pronounced C_2H_4 production at 23°C, where they failed to grow (Fig. 1).

C_2H_4 evolution from cotyledonary segments at different temperatures was almost the same as those of axial segments (Fig. 4). As in CO_2 production (Fig. 2), the C_2H_4 productivity in the cotyledons was also much less and detectable later than that in the axis. Thus the growth of the axis and cotyledons in a seed seems to be stimulated by endogenously evolved CO_2 and C_2H_4 especially from the axis, which consequently leads to breaking of the seed coat. Not only the dormant axes but also the dormant cotyledons did not produce C_2H_4 at 23°C as well as at 13°C, at which their extension growth did not occur.

Effects of C_2H_4 on axis and cotyledon growth: Stimulation of germination in both dormant or impotent seeds of cocklebur by exogenously applied C_2H_4 (*15*, *16*), may be due to the stimulation of growth of both the axis and cotyledons within the seed. This possibility may be supported by a relevant finding that the initial growth of embryonic bean axes is stimulated by exogenous C_2H_4 (*12*). Next, axial and cotyledonary segments excised from dormant and nondormant seeds were allowed to grow at 23°C for 14 and 60 hr, respectively, in the presence of various concentrations of C_2H_4 (Fig. 5).

The dormant axes and cotyledons were notably incapable of growing in the C_2H_4-free air, although the nondormant ones could. Enlargement of dormant

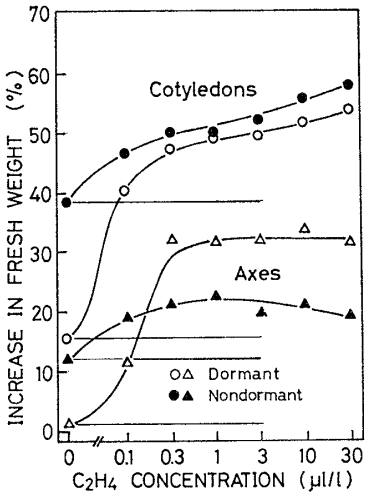

Fig. 5.

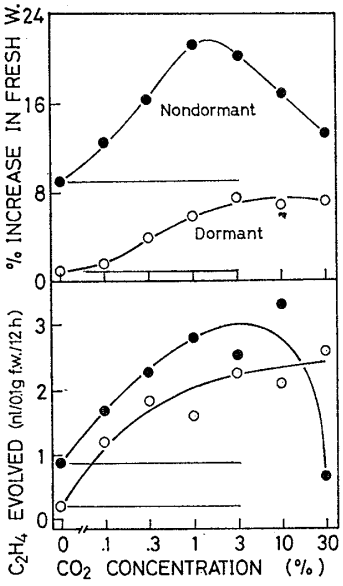

Fig. 6.

Fig. 5. *Dose-response curves of C_2H_4 in extension growth of axial and cotyledonary segments excised from dormant and nondormant cocklebur seeds.* Axial and cotyledonary segments were incubated at 23°C for 14 and 60 hr until weighing. Flasks for no-C_2H_4 were fitted with a side vessel containing $Hg(ClO_4)_2$ solution.
Fig. 6. *Dose-response curves of CO_2 in extension growth and C_2H_4 evolution of dormant and nondormant axial segments.* They were incubated at 23°C for 12 hr. Flasks for no-CO_2 were fitted with a side vessel containing NaOH solution, and in lots for CO_2 concentrations above 3%, the calculated volumes of O_2 were syringed into each flask as needed to maintain 20% O_2 tension.

axes and cotyledons as well as nondormant ones was paralleled by an increase of C_2H_4 concentration in air, except that the C_2H_4 promotion in the nondormant axes was slightly lowered at higher concentration above 1 μl/liter. Interestingly, the dormant axes and cotyledons were much more sensitive to C_2H_4 than the nondormant ones, and this promotion was saturated at a concentration as low as 0.3 μl/liter C_2H_4. These data clearly indicate that the occurrence of seed germination by C_2H_4 is a result of C_2H_4-induced increase of axis and cotyledon enlargements.
Effects of CO_2 on axis and cotyledon growth: Exogenously applied CO_2 sometimes promotes stem (*3, 13, 20*) and coleoptile elongation (*11, 19*), suggesting that stimulation of seed germination by exogenous CO_2 (*2, 29*) may be associated with its accelerating effect on both the axis and cotyledon enlargements. On the other hand, exogenous CO_2 is also known to stimulate C_2H_4 evolution from seeds during a pregermination period (*9, 16*). Therefore, we examined the effects of CO_2 on the enlargement and C_2H_4 production at a different dormant state at 23°C.

As shown in Fig. 6, the dormant axes did not grow at all in the CO_2-free atmosphere, while the nondormant ones did. And the nondormant axes grew more than in the dormant ones, with increasing concentrations of CO_2, the optimum being about 1 and 10% for the nondormant and dormant axes, respectively. Corresponding to these responses, C_2H_4 production from the isolated axes was increased by exogenous CO_2, except for 30% CO_2 in the nondormant ones.

Rijven and Parkash (*25*) reported that CO_2 stimulates the germination of

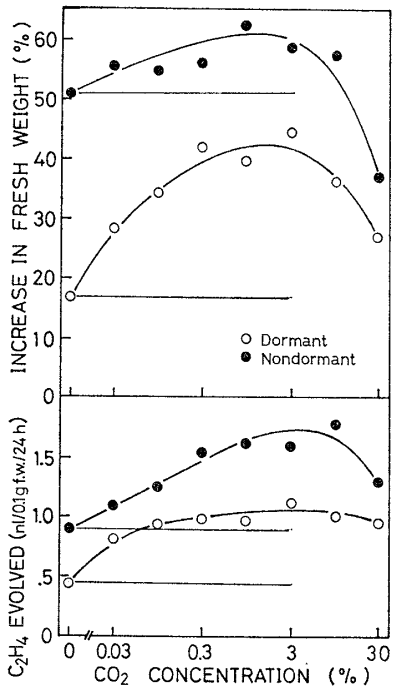

Fig. 7. *Dose-response curves of CO_2 in extension growth and C_2H_4 evolution of dormant and nondormant cotyledonary segments.* Except for the incubation times of 24 hr for C_2H_4 analysis and 72 hr for weighing, other conditions the same as those in Fig. 6.

fenugreek seeds but has no effect on their cotyledon enlargement. Similarly, the growth response of cotyledons to exogenous CO_2 was not as great as that of axes (Fig. 7). In any case, the degree of growth promotion in the dormant axes and cotyledons by CO_2 was much lower than that by C_2H_4 (Fig. 5), and CO_2 application at 1 to 10% produced about 2 times as much C_2H_4 as the CO_2-free atmosphere (Fig. 7). Thus, the question arose whether CO_2 itself stimulates the growth of the organs or causes this effect by accelerating the C_2H_4 production. To answer this question, the next experiment was designed.

Trapping endogenously evolved CO_2 and C_2H_4: Various CO_2- and C_2H_4-trapping treatments in combinations, as shown in Table 2, were conducted. Growth of both organs was greatly reduced by the inclusion of NaOH or $Hg(ClO_4)_2$, the degree being further increased in the presence of both and more striking in the dormant than nondormant ones. In the presence of both absorbents the dormant axes and cotyledons failed to grow normally, unlike the nondormant ones. These data make evident that the growing activities of both organs, especially the dormant ones, depend upon the endogenous C_2H_4 and CO_2 and that the occurrence of relatively large growth in the dormant embryonic axes and cotyledons in the control (no addenda) is probably due to the accumulation of endogenously evolved CO_2 and C_2H_4 within the vials.

The growth-promoting effects of added CO_2 and C_2H_4 were also reduced in the presence of $Hg(ClO_4)_2$ and NaOH, respectively, the degree being greater in the dormant ones. These facts indicate that not only C_2H_4 but also CO_2, which stimulates C_2H_4 production (Fig. 6 and 7), has a specific action in causing the initial extension growth of the axes and cotyledons. Further, the results given in Table 2 suggest a stronger dependency of release from a dormant state in these organs upon C_2H_4 rather than CO_2.

Effects on axis and cotyledon growth of CO_2 and C_2H_4 in combination: Recently, C_2H_4

Table 2 *Effects of CO_2 and C_2H_4 absorbents on the growth of axial and cotyledonary segments from dormant and nondormant cocklebur seeds and on their CO_2- and C_2H_4-stimulated growth*

Treatment	Axes				Cotyledons			
	Dormant		Nondormant		Dormant		Nondormant	
	FW increase	% of control	FW increase	% of control	FW increase	% of control	FW increase	% of control
No addenda	17.5%	100	21.2%	100	28.5%	100	57.9%	100
NaOH	10.2	58	18.6	88	17.6	62	50.3	87
$Hg(ClO_4)_2$	2.7	15	15.8	75	12.5	44	50.2	87
$NaOH+Hg(ClO_4)_2$	2.1	12	12.6	59	7.1	25	46.7	81
1% CO_2	22.8	130	24.0	113	39.8	140	58.2	101
1% $CO_2+Hg(ClO_4)_2$	5.3	30	17.7	84	17.4	61	55.1	95
1 μl/liter C_2H_4	30.8	176	25.8	122	53.2	187	72.4	125
1 μl/liter C_2H_4+NaOH	23.4	137	23.9	113	45.6	160	68.1	118

Fresh weight increments were measured after incubation for 16 hr for the axes and 72 hr for the cotyledons at 23°C.

was found to act synergistically with CO_2 in causing seed germination in some plants (*9*, *16*, *21*, *22*). The results in Table 2 suggest that maximum growth of the excised organs will be achieved in the presence of both CO_2 and C_2H_4. In the experiments given in Fig. 8 and 9, these segments were allowed to grow at 13° and 23°C under various treatments, including 1 μl/liter C_2H_4 plus 3% CO_2.

As shown in Table 2, both axes and cotyledons excised from dormant seeds

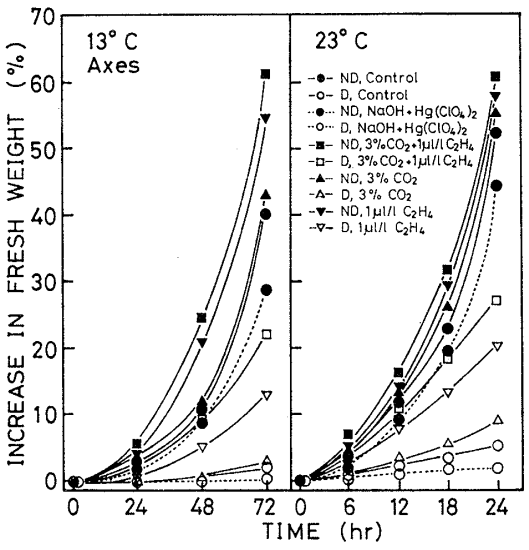

Fig. 8.

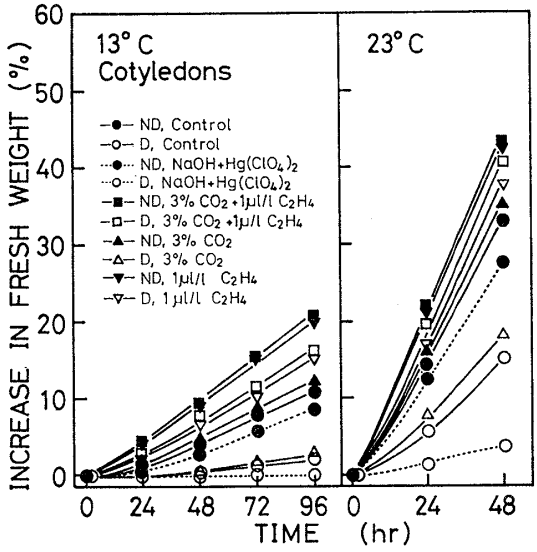

Fig. 9.

Fig. 8. *Growth responses of dormant and nondormant axial segments to CO_2 and C_2H_4 applied singly or in combination at 13° and 23°C.* Details for treatments are given in the figure: D, dormant and ND, nondormant.

Fig 9. *Growth responses of dormant and nondormant cotyledonary segments to CO_2 and C_2H_4 applied singly or in combination at 13° and 23°C.* Details for treatments are given in the figure: D, dormant and ND, nondormant.

Table 3 *Effects of CO_2 (3%) on C_2H_4 evolution in axial and cotyledonary segments from dormant and nondormant cocklebur seeds*

Organ	Temp. (°C)	Incubation time (hr)	C_2H_4 evolution (nl/100 mg fr. wt./hr)			
			Dormant		Nondormant	
			$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$
Axes	13	0–24	0.002	0.002	0.027	0.030
		24–48	0.008	0.009	0.062	0.067
	23	0–12	0.014	0.026	0.125	0.156
		12–21	0.076	0.194	1.284	1.588
Cotyledons	13	0–48	0.002	0.003	0.010	0.013
		48–72	0.008	0.009	0.037	0.041
	23	0–24	0.003	0.014	0.029	0.048
		24–48	0.048	0.113	0.165	0.218

Vials for no-CO_2 were fitted with a side vessel containing NaOH solution. Data are given as the mean C_2H_4 evolution rate in each indicated period.

were nearly incapable of growing even at 23°C when the absorbents for C_2H_4 and CO_2 were both included, whereas, as shown in Fig. 8 and 9, maximal extension growth was obtained regardless of incubation temperature when C_2H_4 was given together with CO_2. In the nondormant segments, however, this combination effect was found only in the axes, the growth of cotyledons depending mainly upon C_2H_4. Furthermore, this CO_2 action was not seen in the dormant axes incubated at 13°C, and contrarily, as already shown in Table 2, the dormant cotyledons were influenced, though slightly, by a concerted action of C_2H_4 and CO_2. Possibly, therefore, the abnormality of the C_2H_4-induced germination—in about the half of the germinated seeds, the seed coat was broken at the cotyledonary side but not at the axial end (*10*)—might result from the action of C_2H_4 on the extension growth of both seed organs. That is, of the critical thrust accumulated during a pregermination period, only one-third or less was derived from the growing cotyledons in the nondormant large seed, but the cotyledon extension stimulated by exogenous C_2H_4 might contribute more substantially to the accumulation of the thrust, thus resulting in the abnormality of the C_2H_4-induced germination.

On the other hand, a condition with no absorbents caused detectable growth of dormant axes and cotyledons at 23°C but not at 13°C. This growth failure in control segments at 13°C may be explainable by the extremely low C_2H_4 productivity at 13°C (Fig. 3 and 4).

CO_2 and C_2H_4 evolution as affected by C_2H_4 and CO_2: Experiments in Table 3 were carried out to examine whether reduction of the CO_2 action at 13°C is associated with the loss of CO_2-stimulated C_2H_4 production. As already described in Fig. 6 and 7, both axes and cotyledons evolved much more C_2H_4, regardless of the dormant status in the presence of 3% CO_2 at 23°C, but not at 13°C. Thus these data suggest that a synergistic interaction of C_2H_4 and CO_2 may result only when CO_2 can stimulate the C_2H_4 production. This possibility is supported also by our previous findings (*13*) that bean hypocotyls did not exhibit such a synergism nor did they exhibit the CO_2-stimulated C_2H_4 production.

On the contrary, there was no enhancement of CO_2 production in the C_2H_4-treated axes and cotyledons independent of incubation temperature and their dormant states (data not shown), although C_2H_4 increased respiration in other storage organs, such as potato tubers (*24*) and iris bulbs (*14*). Thus, the reduction of the C_2H_4 action by NaOH inclusion (Table 2) might not be due to the cancelling of the C_2H_4-stimulated CO_2 production but to the loss of a specific action of endogenous CO_2.

Conclusion

There was a significant difference in growing rate between the axis and cotyledon segments excised from nondormant and dormant cocklebur seeds (Fig. 1). This difference was further magnified when endogenously evolved C_2H_4 and CO_2 were removed: little or no growth of dormant axes and cotyledons occurred at temperatures below 23°C (Table 2, Fig. 8 and 9). Apparently, seed dormancy must be distinguished qualitatively from seed impotency (coat-imposed dormancy) in which an axis and a pair of cotyledons are able to grow, but the seed is unable to germinate due to an insufficient thrust to overcome the seed coat restraint. Therefore, seed dormancy in dicot plants should be defined as a phenomenon resulting from an inability of both the axis and cotyledon to grow, while emergence from dormancy is a process during which these organs regain the ability to grow.

In cocklebur, C_2H_4 is very effective for potentiating the impotent small seed for germination and awaking dormant non-after-ripened small and large seeds (*15*). Even at 13°C exogenous C_2H_4, but not CO_2, caused the dormant segments of both axes and cotyledons to grow and improved the growth rate in the nondormant ones (Fig. 8 and 9). C_2H_4 was found to stimulate the initial growth of the axis and cotyledon, giving the seed enough thrust to overcome the seed coat restraint.

C_2H_4 evolution from nondormant axes and cotyledons increased with an increase of incubation temperature, but dormant axes and cotyledons produced little or no C_2H_4 at temperatures below 23°C (Fig. 3 and 4). Similar to the results with peanut seeds (*17*), however, most of the C_2H_4 evolved from a seed came from the axis (Fig. 3 and 4, Table 3). Comparison of Fig. 3 with Fig. 4 shows that C_2H_4 production from the axis preceeds that from the cotyledons. Therefore, the low C_2H_4 production in dormant seeds is probably due to the extremely low C_2H_4 productivity in the axes.

The effect of C_2H_4 on the extension growth of axes and cotyledons was saturated at a concentration of as low as 0.3 μl/liter (Fig. 5), suggesting that C_2H_4 is a trigger for breaking dormancy in cocklebur seeds. Thus, dormancy in the cocklebur seed is a status in which the axis and cotyledons are prevented from producing C_2H_4.

We have proposed the possibility that there may be two processes for producing C_2H_4 on seed germination: one is the O_2-independent process and the other the O_2-dependent, CO_2-stimulated one (*16*). As shown in Table 3 and Fig. 6 and 7, the enhancement by CO_2 of the growth of dormant axes and cotyledons was not as great as that by C_2H_4, although CO_2 promoted somewhat the production of C_2H_4. Furthermore, there was no significant difference in CO_2 evolution between the dormant and nondormant axes and cotyledons during the early period of incubation (Table 1 and Fig. 2). These findings suggest that repression of a process involving

CO_2 evolution is not the cause of cocklebur seed dormancy, which appears to result from repression of a process influenced by CO_2, such as the O_2-dependent C_2H_4-producing system.

At 33°C, the dormant axes could also produce C_2H_4 with a short lag period (Fig. 3). Therefore, the high temperatures which were very effective for awaking dormant cocklebur seeds and potentiating the small seeds (*15*), may supply a sufficient thrust by releasing the seeds from the state of repressed C_2H_4 production rather than by increasing the growth rate of the axis and cotyledons.

References

(*1*) Andrews, C. J. and G. M. Simpson: Dormancy studies in seed of *Avena fatua*. 6. Germinability of the immature caryopsis. *Can. J. Bot.* 47: 1841–1849 (1969).

(*2*) Ballard, L. A. T.: Studies of dormancy in the seeds of subterranean clover (*Trifolium subterraneum* L.) I. Breaking of dormancy by carbon dioxide and by activated carbon. *Aust. J. Biol. Sci.* 11: 246–260 (1958).

(*3*) Barkley, G. M. and A. C. Leopold: Comparative effects of hydrogen ions, carbon dioxide, and auxin on pea stem segment elongation. *Plant Physiol.* 52: 76–78 (1973).

(*4*) Baskin, J. M. and C. C. Baskin: Germination of *Cyperus inflexus* Muhl. *Bot. Gaz.* 132: 3–9 (1971).

(*5*) Cumming, B. G. and J. R. Hay: Light and dormancy in wild oats (*Avena fatua* L.). *Nature* 182: 609–610 (1958).

(*6*) De la Fuente, P. B. and G. Nicolás: Respiratory activity during germination of seeds of *Cicer arietinum* L. I. Glycolysis and fermentation. *Plant Sci. Letters* 3: 143–148 (1974).

(*7*) Egley, G. H.: Influence of the seed envelope and growth regulators upon seed dormancy in witchweed (*Striga lutea* Lour.). *Ann. Bot.* 36: 755–770 (1972).

(*8*) Esashi, Y. and A. C. Leopold: Physical force in dormacny and germination of *Xanthium* seeds. *Plant Physiol.* 43: 871–876 (1968).

(*9*) Esashi, Y. and A. C. Leopold: Dormancy regulation in subterranean clover seeds by ethylene. ibid. 44: 1470–1472 (1969).

(*10*) Esashi, Y., Y. Hata and H. Katoh: Germination of cocklebur seeds: Interaction between gibberellin, cytokinin, thiourea, KNO_3 and gaseous factors. *Aust. J. Plant Physiol.* 2: No. 4 (in press).

(*11*) Evans, M. L., P. M. Ray and L. Reinhold: Induction of coleoptile elongation by carbon dioxide. *Plant Physiol.* 47: 335–341 (1971).

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

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

(*14*) Kamberbeek, G. A. and A. L. Verlind: Characteristic stimulation by ethylene or respiration in dutch iris bulbs. *Physiol. Plant.* 27: 5–8 (1972).

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

(*16*) Katoh, H. and Y. Esashi: Dormancy and impotency of cocklebur seeds II. Phase sequence in germination process. ibid. 16: 697–706 (1975).

(*17*) Ketring, D. L. and P. W. Morgan: Ethylene as a component of the emanations from germinating peanut seeds and its effect on dormant Virginia-type seeds. *Plant Physiol.* 44: 326–330 (1969).

(*18*) Koller, D., A. Poljakoff-Mayber, A. Berg and T. Diskin: Germination-regulating mechanisms in *Citrullus colocynthis*. *Amer. J. Bot.* 50: 579–603 (1963).

(*19*) Ku, H. S., H. Suge, L. Rappaport and H. K. Pratt: Stimulation of rice coleoptile growth by ethylene. *Planta* 90: 333–339 (1970).
(*20*) Mer, C. L.: Further observations on the effect of carbon dioxide on the growth of etiolated *Avena* seedlings. *Ann. Bot.* 21: 13–22 (1957).
(*21*) Negm, F. B., O. E. Smith and J. Kumamoto: Interaction of carbon dioxide and ethylene in overcoming thermodormancy of lettuce seeds. *Plant Physiol.* 49: 869–872 (1972).
(*22*) Negm, F. B., O. E. Smith and J. Kumamoto: The role of phytochrome in an interaction with ethylene and carbon dioxide in overcoming lettuce seed thermodormancy. ibid. 51: 1089–1094 (1973).
(*23*) Pinfield, N. J. and A. K. Stobart: Hormonal regulation of germination and early seedling development in *Acer pseudoplatanus* L. *Planta* 104: 134–145 (1972).
(*24*) Reid, M. S. and H. K. Pratt: Effects of ethylene on potato tuber respiration. *Plant Physiol.* 49: 252–255 (1972).
(*25*) Rijven, A. H. G. C. and V. Parkash: Cytokinin-induced growth responses by fenugreek cotyledons. ibid. 45: 638–640 (1970).
(*26*) Simmond, J. A. and E. B. Dumbroff: High energy change as a requirement for axis elongation in responses to gibberellic acid and kinetin during stratification of *Acer saccharum* seeds. ibid. 53: 91–95 (1974).
(*27*) Simpson, G. M.: Dormancy studies in seed of *Avena fatua* 4. The role of gibberellin in embryo dormancy. *Canad. J. Bot.* 43: 793–816 (1965).
(*28*) Thornton, N. C.: Factors influencing germination and development of dormancy in cocklebur seeds. *Cont. Boyce Thomp. Inst.* 7: 477–495 (1935).
(*29*) Thornton, N. C.: Carbon dioxide storage. IX. Germination of lettuce seeds at high temperatures in both light and darkness. ibid. 8: 25–40 (1936).
(*30*) Toole, V. K., W. K. Bailey and E. H. Toole: Factors influencing dormancy of peanut seeds. *Plant Physiol.* 39: 822–832 (1964).
(*31*) Villiers, T. A. and P. F. Wareing: Dormancy in fruits of *Fraxinux excelsior* L. *J. Exp. Bot.* 15: 359–367 (1964).
(*32*) Wareing, P. F. and H. A. Foda: Growth inhibitors and dormancy in *Xanthium* seed. *Physiol. Plant.* 10: 266–280 (1957).
(*33*) Wareing, P. F. and P. F. Saunders: Hormones and dormancy. *Ann. Rev. Plant Physiol.* 22: 261–288 (1971).
(*34*) Webb, D. P. and P. F. Wareing: Seed dormancy in *Acer pseudoplatanus* L.: The role of the covering structures. *J. Exp. Bot.* 23: 813–829 (1972).