Plant Physiol. (1981) 67, 503–508
0032-0889/81/67/0503/06/$00.50/0

Cyanide-sensitive and Cyanide-resistant Respiration in the Germination of Cocklebur Seeds

Received for publication November 13, 1979 and in revised form September 17, 1980

YOHJI ESASHI, YUUKO SAKAI, AND RYOHKO USHIZAWA
Department of Biological Science, Tohoku University, Kawauchi, Sendai 980, Japan

ABSTRACT

Interrelation between the CN-sensitive cytochrome path and the CN-resistant, benzohydroxamic acid (BHM)-sensitive, or *n*-propylgallate (nPG)-sensitive alternative path in seed respiration during germination was examined using the nondormant upper and lower seeds of *Xanthium pensylvanicum* Wallr. The operation of both paths was required not only for normal germination of the lower seed but also for KCN- or NaN_3-induced germination of both. From the sensitivity to BHM of the germination response, it became obvious that the alternative path exerts its physiological activity as soon as it develops during the early period of water imbibition. Pretreatments with KCN and NaN_3 for promoting germination, strikingly decreased only the engagement of the cytochrome path in the subsequent respiration without affecting that of the alternative path. Nevertheless, no germination occurred without the operation of the cytochrome path. This suggested that excess operation of the cytochrome path is detrimental to germination, being maximal following the BHM-sensitive phase.

The alternative and cytochrome paths operated in a proportion of 1 to 5 in a period just before the lower seeds started to germinate. However, there was little difference between the upper seeds of relatively low germination potential and the lower seeds of relatively high potential with respect to both the developmental pattern of the alternative path and the balance of the alternative and cytochrome paths. The higher germination potential of the lower seeds may be related to their high capacities for the alternative path.

Conventional respiratory inhibitors, such as KCN, NaN_3, and NH_2OH, are known to stimulate the germination of cocklebur seeds (6) and other seeds (9, 10, 14, 16). Taylorson and Hendricks (22) attributed the germination-promoting action of KCN to the formation of β-cyanoalanine by which seed germination is accelerated. Later Hendricks and Taylorson (11) explained such an action of NH_2OH and KN_3 based on the H_2O_2-sparing action through catalase inhibition by which the pentose phosphate pathway, supplying the reducing force necessary for seed germination to occur, is activated. However, we could not obtain any evidence in support of their views: β-cyanoalanine did not promote the germination of cocklebur seeds (6) and the degree of catalase inhibition by these inhibitors did not reflect their effectiveness on germination (8).

In a previous paper (7), we reported that a CN-resistant, alternative respiration path engages in the germination process of cocklebur seeds and is activated by C_2H_4 which stimulates germination. It has also been shown that CN and N_3 prompt the diversion of electron transport from the Cyt path to the alternative path (23). These results led to the possibility that the germination-promoting effects of these respiratory inhibitors may be due to the activation of the alternative path, similar to the case of C_2H_4, and, conversely, to the question of whether or not the operation of the CN-sensitive, Cyt path is essential for seed germination to occur. Even though the operation of both paths is a prerequisite for the occurrence of seed germination, we do not yet know how the Cyt and alternative paths are mutually related to the germination process. Moreover, a different engagement of each path may explain the difference in germinability of the upper and lower seeds within a cocklebur fruit.

MATERIALS AND METHODS

Nondormant upper and lower seeds of *Xanthium pensylvanicum* Wallr., stored at 8 C after being harvested in the fall of 1977, were used in all experiments. Except where otherwise mentioned, all experiments were carried out at 23 C according to the previously described procedures (7). The growth and O_2 uptake in the decoated seed tissues of cocklebur seeds were significantly inhibited by BHM,[1] as an inhibitor of the alternative respiration (17), at concentrations of a few mM [far lower than those required in the intact seed (7)], suggesting the low permeability of the cocklebur seed coat, which contains a layer of endosperm cells, to BHM. Therefore, very high concentrations of BHM, ranging from 20 to 100 mM, were supplied throughout the study presented here in which only whole seeds were used. Also, nPG, recently reported to be an inhibitor of alternative respiration (18), was administered. In a preliminary experiment, on the other hand, the respiration-inhibiting effect of KCN solutions, buffered to pH 7.2, remained nearly constant for 4, 7, and 12 h at 5, 10, and 20 mM, respectively. KCN was applied at the high concentrations of 20 or 30 mM in order to keep its effective concentration throughout experimental periods ranging from 6 to 9 h. We assumed that the CN effect was primarily on the Cyt pathway, although at such high concentrations it is recognized that other oxidases may be inhibited.

RESULTS

O_2 Uptake of Upper and Lower Seeds in Presence of BHM and KCN. In the first place, we examined whether the sensitivities for seed respiration to BHM, an inhibitor of the alternative path, and to KCN, an inhibitor of the Cyt path, were similar for upper and lower seeds (Fig. 1). Thirty unimbibed upper and 22 lower seeds, of the same total weight, were arranged on a sheet of filter paper lining a manometric vessel and wetted with 1 ml test solution, after being presoaked with the same test solutions for 20 min.

Regardless of seed type, O_2 uptake rate in water controls increased rapidly and linearly during the first few h after water imbibition and, afterward, the rate of increase declined gradually. At all times during water imbibition, however, the lower seed

---

[1] Abbreviations: BHM, benzohydroxamic acid; nPG: *n*-propylgallate.

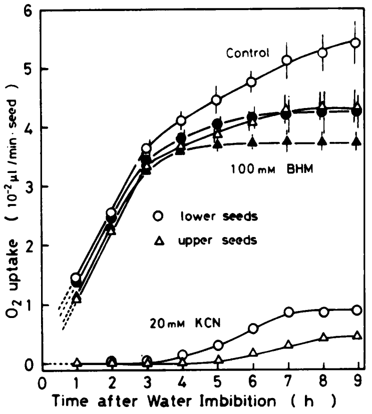

FIG. 1. Time courses of respiration of upper and lower cocklebur seeds in the absence or presence of 20 mM KCN or 100 mM BHM. Vertical bars show standard deviations of means of triplicate determinations.

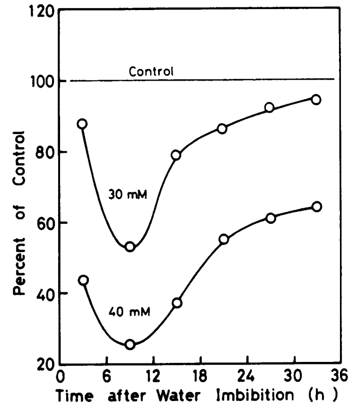

FIG. 2. BHM-sensitive phase during germination process. Unimbibed lower cocklebur seeds were treated with 30 or 40 mM BHM solution during a 6-h period beginning 0, 6, 12, 18, 24, or 30 h after the start of water imbibition at 23 C, then rinsed several times with water, and returned to water for germination test. Germinated seeds among those presoaked for 24- or 30-h were discarded prior to BHM application. Per cent of control germination after 70 h is shown in the middle of each period of treatment; untreated control seeds germinated 76.8%. Differences of 9.6% or more between treated and control seeds are significant at $P = 0.01$.

exhibited a higher O_2 uptake rate than the upper ones did and the difference became more significant after 5 h imbibition. This difference was seen in the presence of both BHM and KCN. From these results, it is evident that the property of aerobic respiration systems is almost the same in the upper and lower seeds.

As reported in a previous paper (7), sensitivity for seed respiration to BHM was not demonstrated during an earlier period of water imbibition: the inhibition of respiration by BHM was not achieved until 5 to 6 h and 6 to 7 h soaking for lower and upper seeds, respectively. This could be due to a BHM-sensitive respiration developing only after 5 to 7 h soaking or due to a lag in penetration and buildup of BHM in the seed. On the other hand, the CN inhibition of respiration was complete during the early period of imbibition but became incomplete after 5 to 6 h imbibition. The results seem to indicate that a CN-resistant component, probably the alternative path, does not function during the first 3 to 4 h of imbibition, regardless of seed type, but develops during the early period of water imbibition, earlier in the lower seed than in the upper one.

BHM Sensitivity During Germination Process. Experiments in Figure 2 were designed to determine which period during water imbibition was most sensitive to BHM for germination inhibition. Unimbibed lower cocklebur seeds, capable of germinating at 23 C, were employed inasmuch as there was no apparent difference in respiration characteristics between the lower and upper ones. They were treated with 30 or 40 mM BHM for a 6-h interval at different times after the start of water imbibition at 23 C.

In either case, maximum inhibition by BHM was caused when BHM was applied between 6 and 12 h of water imbibition, which indicates that the BHM-sensitive component, probably the alternative path, plays a significant role in the germination process during that time period.

KCN and NaN_3 Sensitivity During Germination Process. To determine the time sensitive to KCN and NaN_3 during a water-imbibition period, unimbibed upper seeds were treated as in Figure 2. Both KCN and NaN_3 were slightly effective in stimulating germination even when applied during an initial 6 h water imbibition, but they were more effective in the application after 12 h, which follows the BHM-sensitive phase (Fig. 3).

Respiration Inhibition by KCN and NaN_3. In Figure 1, the inhibition of respiration by 20 mM KCN became incomplete with time. This incomplete CN inhibition is not attributed to the distillation loss of CN from the solution into alkali in the sidearm within an indicated experimental period. Nevertheless, there is a possibility that this phenomenon may be due to the decreasing concentration of CN inside the seed as a result of enzymic conversion of CN and, thus, not due to the development of the CN-resistant path. Therefore, the dose-response relations for KCN and NaN_3 in the respiration of upper cocklebur seeds were examined and shown in Figures 4 and 5, respectively. Prior to O_2 measurements, the seeds were presoaked for 20 h to allow them to develop the alternative path. In either case, O_2 uptake rate decreased as the concentrations of KCN and NaN_3 were increased, but the O_2 uptake rate did not fall to zero, suggesting that the incomplete inhibition of respiration by KCN is not due to its decreasing concentration. Cocklebur seeds exhibited CN- and N_3-insensitive respiration of about 15 to 20% of total respiration capacity, which probably corresponded to the proportion of the BHM-sensitive respiration in Figure 1.

Necessity of Cyt Path for Germination. Although KCN and NaN_3 strongly inhibited the respiration of cocklebur seeds (Figs. 4 and 5), they promoted germination, which implies that the operation of the Cyt path is unnecessary for the induction of germination. However, the promotion of seed germination by

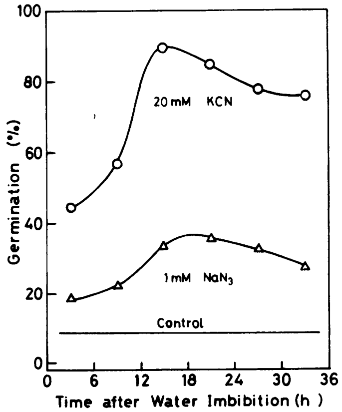

FIG. 3. KCN- and NaN_3-sensitive phases during germination process. Unimbibed upper cocklebur seeds were exposed to 20 mM KCN or 1 mM NaN_3 during a 6-h period beginning 0, 6, 12, 18, 24, or 30 h after the start of water imbibition at 23 C, then rinsed several times with water, and returned to water for germination test. Germination percentages after 70 h are shown in the middle of each period of treatment and control seeds were imbibed but not treated with KCN or NaN_3. Differences of 7.4% or more between treated and control seeds are significant at $P = 0.01$.

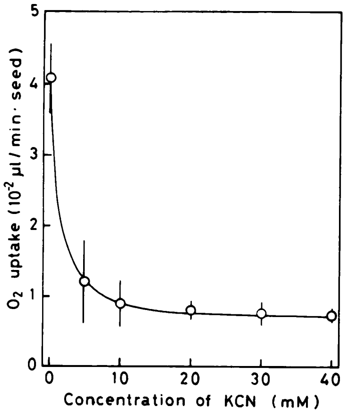

FIG. 4. Dose-response curve for KCN in the respiration of 20-h presoaked upper cocklebur seeds at 23 C. Data are shown by values with standard deviations obtained during a period from 3.5 to 4 h after incubation.

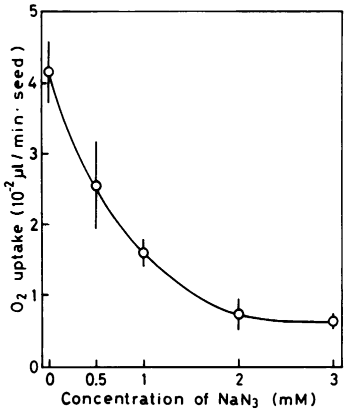

FIG. 5. Dose-response curve for NaN_3 in the respiration of 20-h presoaked upper cocklebur seeds at 23 C. Data are shown by values with standard deviations obtained during a period from 3.5 to 4 h after incubation.

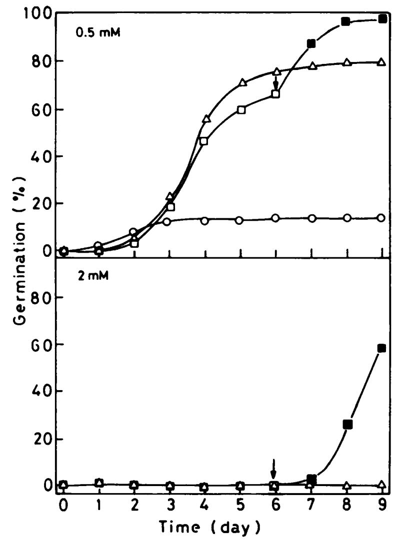

FIG. 6. Effects of renewal of applied NaN_3 on the germination of upper cocklebur seeds at 23 C. Unimbibed seeds were subjected continuously (△) to 0.5 or 2 mM NaN_3 or to daily renewals of these inhibitor concentrations during 6 days (□). The latter ones were rinsed with water (at arrows) and then returned to water for germination test. A sample of seeds was maintained only in water as a blank (○).

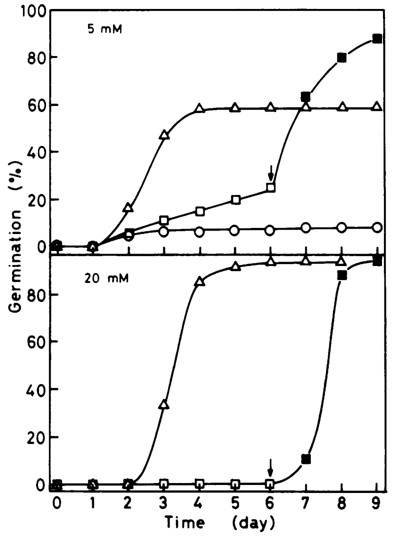

FIG. 7. Effects of renewal of applied KCN on the germination of upper cocklebur seeds at 23 C. Unimbibed seeds were subjected continuously (△) to 5 or 20 mM KCN or to daily renewals of these inhibitor concentrations during 6 days (□). The latter ones were rinsed with water (at arrows) and then returned to water for germination test. A sample of seeds was maintained only in water as a blank (○).

KCN of 20 mM, at which concentration the Cyt respiration is completely prevented (Fig. 4), may result from the reduction of CN concentration inside the seed due to the conversion of CN to β-cyanoalanine (22) inasmuch as NaN_3 at 2 mM, which fully inhibits the Cyt respiration (Fig. 5), fails to stimulate the germination (6). Therefore, KCN and NaN_3 solutions at 5 or 20 mM and 0.5 or 2 mM, respectively, were renewed daily and then, after 6 days, the KCN and NaN_3 solutions washed away several times with water, after which seeds were returned to water (Fig. 6). Control seeds were continuously in contact with the original test solutions throughout the experimental period. In NaN_3-treated seeds (Fig. 6), 0.5 mM NaN_3 induced germination, regardless of whether it was applied with or without renewal. Although NaN_3 at 2 mM did not induce germination in either case, the seeds which had been subjected to the daily application of 2 mM NaN_3 germinated rapidly after being rinsed with water on day 6.

On the other hand, the repeated applications of KCN, unlike NaN_3, induced germination only at a far lower rate than did the control with continuous exposure to nonrenewed KCN solutions, when KCN concentration was 5 mM, resulting in only the incomplete inhibition of respiration (Fig. 7). In the presence of 20 mM KCN, cocklebur seeds began to germinate after 2 days unless KCN was renewed and showed about 100% germination at day 5. When the KCN solution was renewed the seeds were incapable of germinating until KCN was rinsed away. These results may indicate that no germination process advances when the N_3- and CN-sensitive respiration paths, mostly the Cyt path, are completely blocked. This conclusion assumes that the high concentrations of KCN or NaN_3 completely blocked respiration, especially when renewed, and that this mediated the germination response rather than some other less specific effect of the inhibitors expressed at high concentrations.

Respiration of Seeds Pretreated with KCN and NaN_3. Pretreatments with high concentrations of NaN_3 and KCN were effective in stimulating cockebur seed germination (Figs. 6 and 7). Table I compares the respiration systems of KCN- or NaN_3-pretreated seeds with those of untreated ones. To develop the alternative path, upper cockebur seeds were presoaked at 23 C for 20 h prior

Table I. *Effects of KCN and NaN_3 Pretreatments on Respiration of Upper Cocklebur Seeds in Absence or Presence of KCN or BHM*

Seeds presoaked for 20 h then were pretreated with 30 mM KCN or 1 mM NaN_3 for 16 h, rinsed thoroughly with water, and arranged in a manometric vessel. Control seeds were maintained only in water. Data with ± SD were taken after 5 h incubation.

Pretreatment	O_2 Uptake				
	H_2O	20 mM KCN	Inhibition	100 mM BHM	Inhibition
	10^{-2} *μl/min·seed*		%	10^{-2} *μl/min·seed*	%
H_2O	3.95 ± 0.28	0.75 ± 0.05	81.0	3.29 ± 0.28	16.7
30 mM KCN	1.96 ± 0.22	0.73 ± 0.09	62.8	0.57 ± 0.10	70.9
H_2O	4.07 ± 0.43	0.76 ± 0.10	81.3	3.20 ± 0.31	21.4
1 mM NaN_3	1.88 ± 0.38	0.75 ± 0.08	60.1	0.36 ± 0.08	81.9

Table II. *Effect of BHM on KCN-induced Cocklebur Seed Germination*

Unimbibed and 33-h presoaked upper cocklebur seeds were previously treated with 30 mM KCN for 48 or 15 h, rinsed thoroughly with water, and then contacted with BHM at indicated concentrations for 9 or 48 h, respectively. They were returned to water for germination test after being again rinsed. Data were taken 4 days after the end of KCN treatments. Seeds pretreated with only BHM did not germinate at all.

Seed	Treatment		Germination
	A	B	
			%
Unimbibed[a]	H_2O	H_2O	10.8
	30 mM KCN	H_2O	100
	30 mM KCN	50 mM BHM	52.6
	30 mM KCN	100 mM BHM	22.5
33-h preimbibed[b]	H_2O	H_2O	0
	30 mM KCN	H_2O	100
	30 mM KCN	20 mM BHM	7.6
	30 mM KCN	50 mM BHM	0

[a] Treatment A, first 48 h; treatment B, next 9 h.

[b] Treatment A, first 15 h; treatment B, next 48 h.

to O_2 measurement and then exposed to 30 mM KCN or 1 mM NaN_3, these concentrations being most effective in inducing cocklebur seed germination (6). In the absence of BHM or KCN, the O_2 uptake rates of pretreated seeds were approximately half that of the water controls. The inhibitory effect of BHM was markedly increased by pretreatment with KCN or NaN_3, which may imply the increased engagement of the alternative path by KCN and NaN_3 pretreatments. The degrees of traffic through the CN-resistant path, as measured in the presence of KCN, although probably containing the residual component insensitive to both KCN and BHM or nPG (Table V), were almost the same between pretreated and untreated ones, thus suggesting that KCN and NaN_3 pretreatments do not increase the extent of engagement of the CN-resistant, alternative path, but increase the balance of the alternative path to the Cyt path by decreasing the extent of flux through the latter. The increase in BHM inhibition of O_2 uptake by the pretreatments may be partly because some CN and N_3 still remain in the pretreated seeds following washing, for it is known that BHM and KCN or NaN_3 in combination almost completely suppress the respiration of cocklebur seeds (unpublished data) (Table V).

Importance of Alternative Path in KCN and NaN_3 Effects. As described in Figures 6 and 7, the operation of the Cyt path was concluded to be required for KCN and NaN_3 to be effective in stimulating cocklebur-seed germination. Table II shows not only the operation of the Cyt path but also that of the alternative path are absolutely essential for the KCN-induced germination. Unimbibed or 33-h preimbibed upper cocklebur seeds were pretreated with 30 mM KCN for 48 or 15 h, rinsed several times with water, and then contacted with BHM of indicated concentrations for 9 or 48 h, respectively. Regardless of whether seeds were preimbibed or not, KCN-pretreated seeds exhibited 100% germination, but the KCN-induced germination was prevented by subsequently applied BHM, the degree of the inhibition being in proportion to its concentration and to the duration of its application.

Different Balance between Cyt and Alternative Paths in Upper and Lower Seeds. In Figure 1, there was no appreciable quantitative or qualitative difference in the developmental pattern of the alternative path between upper and lower cocklebur seeds. From Table I, however, it was suggested that some traffic balance of the Cyt and alternative paths may be essential for the occurrence of cocklebur seed germination, which further implies that a difference in germination potential between the upper and lower seeds may be attributable to a different balance in engagement between the Cyt and alternative paths. In an experiment shown in Table III, therefore, 16-h presoaked upper and lower cocklebur seeds, unlike the case in Figure 1 in which unimbibed seeds were used, were placed in manometric vessels in the presence of either 20 mM KCN or 100 mM BHM. In this case, 16 h presoaking was adopted as a period during which the seeds developed sufficiently the alternative path but could not germinate. Afterwards, the seeds used were rinsed with water and then transferred onto a water substratum in Petri dishes for germination test (Table IV). Contrary to expectation, there was no significant difference in the proportion of flux via the alternative path to that via the Cyt path between upper and lower seeds, even at a time just before the latter began to germinate. However, the O_2 uptake rates of the lower seeds were 1.57 (for water control), 1.56 (for KCN), and 1.59 (for BHM) times greater than those of the upper ones, although the lower seeds used here were only 1.43 times heavier in a fresh weight basis than were the upper ones. These results indicate that the lower seeds have a slightly greater capacity for respiration through each path than did the upper ones. Moreover, the germination of the lower seeds was also stimulated by pretreatment with KCN (Table IV).

Similarity in the ratio of the alternative path flux to the Cyt path flux between the upper and lower seeds was further established from the data shown in Table V. They were similarly presoaked and respired in the presence of 20 mM KCN alone or combined with either 100 mM BHM or 15 mM nPG. The fluxes via the residual component insentitive to both KCN and BHM or nPG accounted for only 3 to 4% of the O_2 uptake in the untreated seeds, regardless of the type of seeds. The flux via the Cyt path accounted for about 80% of the total respiration in either seed. The remaining 16 to 17% fluxes were via the alternative path. As in Table III, the net fluxes via each path were much greater in the lower seed than in the upper one. However, the alternative/Cyt (V_{alt}/V_{cyt}) ratio was similar in the upper and lower seeds, being 0.19.

DISCUSSION

Previous work has reported that there is a CN-resistant, alternative path in the respiration system of cocklebur seeds, and its

Table III. *Comparison of Respiration in Absence or Presence of KCN or BHM between Upper and Lower Cocklebur Seeds*

Seeds presoaked for 16 h at 23 C were used and data with ± SD were taken 5 h after incubation.

Seed	O₂ Uptake				
	H_2O	20 mM KCN	Inhibition	100 mM BHM	Inhibition
	10^{-2} *µl/min·seed*		%	10^{-2} *µl/min·seed*	%
Upper	4.11 ± 0.39	0.79 ± 0.26	80.8	3.08 ± 0.43	25.1
Lower	6.47 ± 0.57	1.23 ± 0.41	81.0	4.89 ± 0.52	24.4

Table IV. *Comparison of Germination Response to 20 mM KCN and 100 mM BHM between Upper and Lower Seeds*

The same seeds as those used in Table III were contacted with test solutions for a further 4-h period, 9 h in total, and returned to water for germination test after being rinsed with water. Data were taken 42 h after the start of water imbibition.

Seed	Germination		
	H_2O	20 mM KCN	100 mM BHM
		%	
Upper	0	13.3	0
Lower	31.0	100.0	0

operation is required for germination (7). Figure 1 and Table V show the existence of the CN-sensitive, Cyt path besides the alternative path in this seed. As shown in Figures 4 and 5 and Tables III and V, respiration via the CN- or N_3-sensitive path accounted for about 80% of the total respiration of the upper cocklebur seed. The CN- or N_3-sensitive path may partly include other oxidases than the Cyt oxidase inasmuch as the higher concentrations of KCN and NaN_3 used here are known to inhibit a wide range of oxidases. Nevertheless, this would not deny that a major electron transport system in this seed is the Cyt path. The complete inhibition of respiration was not caused by KCN and NaN_3 even though their concentrations were raised (Figs. 4 and 5); 17 to 20% of the total respiration was insensitive to CN and N_3. Conversely, 100 mM BHM inhibited about 20% of O_2 uptake in the upper seeds not pretreated with KCN or NaN_3 (Table I). In Table V, it was shown that the flux via the alternative path accounts for about 16% of the total respiration. Thus, the proportion of the alternative path to the cytochrome path in the electron traffic is approximately 1:5.

It is known that soybean seeds respire via the alternative path in an initial period of water imbibition (24). In the seed of *Cicer arietinum*, the alternative path is known to develop during a water-imbibition period (1). Also, the alternative path of cocklebur seeds appeared at 5 to 7 h after the start of water imbibition (ref. 7; Fig. 1), and it contributed heavily to the germination process at the earliest stage (Fig. 2). Because it has been reported that O_2 is unnecessary for cocklebur seed germination during the first 8 h of a water-imbibition period (13) and that the phase most sensitive to BHM is located in a period ranging from h 6 to 12 of the water imbibition, one is led to believe that the O_2 which contributes initially to advance the germination process may be consumed mainly in the alternative path. However, the operation of the alternative path is also required for KCN- and NaN_3-induced germination (Table II). Accordingly, the alternative path would be a respiratory system which performs an important function in the first aerobic step of processes in seed germination. If so, the present result can also be reconciled with those obtained for soybean seeds (24).

In plant tissues having the alternative path, KCN is known to increase the engagement of the alternative path and, consequently, to promote respiration (19, 21). In the present case, pretreatments with KCN and NaN_3 did not result in any increase of respiration nor enhance a flux via the alternative path (Table I). Therefore, it is not likely that the primary action of KCN and NaN_3 in germination stimulation, unlike C_2H_4 (7), is caused through activation of the alternative path.

The germination-promoting action of KCN and NaN_3 did not appear under conditions in which the O_2 uptake via the Cyt path could not occur (Figs. 6 and 7). This assumes that, at high KCN and NaN_3 concentrations, the Cyt path is completely blocked and yet no other effect of the inhibitors occurs to block germination. The conclusions about the Cyt path drawn from Figures 6 and 7 indicate that cocklebur seeds require the operation of the CN-sensitive, Cyt path in addition to that of the alternative path to germinate. Nevertheless, pretreatments with KCN and NaN_3 at lower concentrations (than those which blocked germination in Figs. 6 and 7) brought about a decrease in the traffic capacity of the Cyt path but not an increase in that of the alternative path (Table I) and, as a result, these pretreatments raised the proportion of flux via the alternative path to that of the Cyt path. It is likely that a very high operation of the Cyt path may be inhibitory to germination, and the germination-promoting action of KCN and NaN_3 may result from the decreased flux of the Cyt path.

The lower germination potential of upper cocklebur seeds has been attributed to the existence of the germination-inhibiting system, which was inferred from the germination-inducing effects of anaerobiosis and chilling (3–5). However, both the anaerobiosis and chilling were ineffective in an earlier period of water imbibition, becoming effective with time (3, 4), and the later exhibited the maximum effect after 2 weeks (4). Similarly, the effects of KCN and NaN_3 on germination increased with time of water imbibition prior to their application (Fig. 3) and the effects of KCN and NaN_3 treatments for 10 days reached maximum between days 8 and 16 (6). These findings imply that the supposed germination-inhibiting system may be involved in excessive operation of the Cyt path.

The germination-promoting effect of KCN and NaN_3 was most striking when they were applied during a period following the BHM-sensitive phase (Fig. 3). Therefore, the detrimental action of the excessively operating Cyt path may appear only after the alternative path exerts its physiological activity on germination. Inasmuch as the germination-promoting action of KCN and NaN_3 occurred over a period of 3 weeks after the start of water imbibition (6), the over-operation of the Cyt path is considered to continue for at least 3 weeks or longer.

Although the germination potential of the lower cocklebur seed was very high compared to that of the upper one (2), there was little difference between the lower and upper seeds not only in the developmental pattern of the alternative path (Fig. 1) but also in the proportion in flux of the alternative path to the Cyt path (Tables III and V). Moreover, the higher germination potential of the lower seed could not be attributed to a lower activity in the germination-inhibiting system because KCN could promote the germination of the lower seed as well as the upper one (Table V). The germination of the lower seeds was inhibited by BHM (7; Fig. 2; Table IV) and their traffic capacities for both the Cyt and alternative paths were about 1.6 times greater than those of the upper ones (Tables III and V). These facts suggest that the higher germination potential of the lower seed may arise from the high

Table V. *Comparison of Respiration in Absence or Presence of Inhibitors between Upper and Lower Cocklebur Seeds*

Seeds presoaked at 23 C for 16 h were used and data were shown by the means ± SD of values obtained between 4 and 6 h after mounting with 30 mM KCN, 100 mM BHM, and 15 mM nPG. V_{cyt} and V_{alt} were tentatively given as differences between untreated and KCN and between KCN and KCN + BHM or nPG.

Seed	O$_2$ Uptake					V_{alt}/V_{cyt}
	Untreated	KCN	KCN + BHM	V_{cyt}	V_{alt}	
			10^{-2} μl/min·seed			*ratio*
Upper	4.33 ± 0.18	0.84 ± 0.24	0.18 ± 0.13	3.49	0.66	0.19
Lower	6.30 ± 0.51	1.21 ± 0.35	0.23 ± 0.14	5.09	0.98	0.19
Upper	3.92 ± 0.37	0.72 ± 0.15	0.14 ± 0.09[a]	3.11	0.58	0.19
Lower	6.03 ± 0.48	1.14 ± 0.26	0.20 ± 0.12[a]	4.99	0.94	0.19

[a] Values are for KCN + nPG (not KCN + BHM).

traffic flux via the alternative path, although the flux via the Cyt path was also great. Such a property of the lower seed is probably associated with its higher ability to produce C_2H_4 than that of the upper seed (12) because C_2H_4 is known to activate the alternative path (7).

LITERATURE CITED

1. Burguillo PF, G Nicolás 1977 Appearance of an alternative pathway cyanide-resistant during germination of seeds of *Cicer arietinum*. Plant Physiol 60: 524–527
2. Esashi Y, AC Leopold 1968 Physical force in dormancy and germination of *Xanthium* seeds. Plant Physiol 43: 871–876
3. Esashi Y, Y Ohhara 1977 Enhancement by low temperatures of the anaerobic induction of cocklebur seed germination. Aust J Plant Physiol 4: 849–855
4. Esashi Y, Y Tsukada, Y Ohhara 1978 Interrelation between low temperature and anaerobiosis in the induction of germination of cocklebur seed. Aust J Plant Physiol 5: 337–345
5. Esashi Y, Y Tsukada 1978 Thermoperiodism in cocklebur seed germination. Plant Physiol 61: 437–441
6. Esashi Y, Y Ohhara, M Okazaki, K Hishinuma 1979 Control of cocklebur seed germination by nitrogenous compounds: nitrite, nitrate, hydroxylamine, thiourea, azide, and cyanide. Plant Cell Physiol 20: 349–361
7. Esashi Y, S Wakabayashi, Y Tsukada, S Satoh 1979 Possible involvement of the alternative respiration system in the C_2H_4-stimulated germination of cocklebur seeds. Plant Physiol 63: 1039–1043
8. Esashi Y, Y Sakai, R Ushizawa, S Tazaki 1979 Catalase is not involved in control of germination of cocklebur seeds. Aust J Plant Physiol 6: 425–429
9. Hendricks SB, RB Taylorson 1972 Promotion of seed germination by nitrates and cyanides. Nature 237: 169–170
10. Hendricks SB, RB Taylorson 1974 Promotion of seed germination by nitrate, nitrite, hydroxylamine, and ammonium salts. Plant Physiol 54: 304–309
11. Hendricks SB, RB Taylorson 1975 Breaking of seed dormancy by catalase inhibition. Proc Natl Acad Sci USA 72: 306–309
12. Katoh H, Y Esashi 1975 Dormancy and impotency of cocklebur seeds. I. CO_2, C_2H_4, O_2, and high temperature. Plant Cell Physiol 16: 687–696
13. Katoh H, Y Esashi 1975 Dormancy and impotency of cocklebur seeds. II. Phase sequence in germination process. Plant Cell Physiol 16: 697–706
14. Roberts EH 1964 The distribution of oxidation-reduction enzymes and the effects of respiratory inhibitors and oxidising agents on dormancy in rice seed. Physiol Plant 17: 14–29
15. Roberts EH 1973 Oxidative processes and the control of seed germination. *In* W Heydecker, ed, Seed Ecology. Butterworth, London, pp 189–218
16. Rollin P 1975 Influence de quelques inhibiteurs sur la respiration et la germination des akènes de Bidens radiata. Physiol Vég 13: 362–382
17. Schonbaum GR, WD Bonner, Jr, BT Strey, JT Bahr 1971 Specific inhibition of the cyanide-insensitive respiratory pathway in plant mitochondria by hydroxamic acids. Plant Physiol 47: 124–128
18. Siedow JN, ME Girvin 1980 Alternative respiratory pathway. Its role in seed respiration and its inhibition by propyl gallate. Plant Physiol 66: 669–674
19. Solomos T, GG Laties 1975 The mechanism of ethylene and cyanide action in triggering in the rise in respiration in potato tubers. Plant Physiol 55: 73–78
20. Solomos T, GG Laties 1976 Induction by ethylene of cyanide-resistant respiration. Biochem Biophys Res Commun 70: 663–671
21. Solomos T, GG Laties 1976 Effects of cyanide and ethylene on the respiration of cyanide-sensitive and cyanide-resistant plant tissues. Plant Physiol 58: 47–50
22. Taylorson RB, SB Hendricks 1973 Promotion of seed germination by cyanide. Plant Physiol 52: 23–27
23. Theologis A, GG Laties 1978 Antimycin-insensitive cytochrome-mediated respiration in fresh and aged potato slices. Plant Physiol 62: 238–242
24. Yentur S, AC Leopold 1976 Respiratory transition during seed germination. Plant Physiol 57: 274–276