Plant Physiol. (1988) 87, 655–659
0032-0889/88/87/0655/05/$01.00/0

Cyanide-Resistant Respiration in Light- and Dark-Grown Soybean Cotyledons[1]

Received for publication November 23, 1987 and in revised form February 5, 1988

ABU SESAY[2], CECIL R. STEWART*, AND RICHARD SHIBLES
Department of Agronomy (A.S., R.S.), *Department of Botany* (C.R.S.), *and Department of Plant Pathology* (C.R.S.), *Iowa State University, Ames, Iowa 50011*

ABSTRACT

Measurements of respiration were made on intact tissue and mitochondria isolated from soybean (*Glycine max* [L.] Merr. cv 'Corsoy') cotyledons from seedlings of different ages grown in light and darkness. Effects of cyanide (KCN) and salicylhydroxamic acid (SHAM) on O_2 uptake rates were determined. O_2 uptake was faster in light-grown tissue and was inhibited by both KCN and SHAM in all except light-grown tissue older than 9 days. Both inhibitors stimulated O_2 uptake in tissues more than 9 days old. Mitochondria in which O_2 uptake was coupled to ATP synthesis were isolated from all tissues. O_2 uptake by mitochondrial preparations from light- and dark-grown cotyledons was equally sensitive to KCN. Similarly, age did not affect KCN sensitivity, but sensitivity to SHAM declined with age both in the presence and absence of KCN. Estimated capacities of the cytochrome and alternative pathways of the mitochondrial preparations indicated considerably larger cytochrome than alternative pathway capacities. The cytochrome pathway capacities paralleled the state 3 mitochondrial respiration rates, which increased from day 5 to day 7 then declined thereafter. The alternative pathway capacities were not affected by light. The uncoupler, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone (FCCP), increased the flow of electrons through the cytochrome pathway at the expense of flow through the alternative pathway in isolated mitochondria. However, the combined capacities did not exceed the rate in the presence of FCCP. The results are interpreted to indicate that the stimulation of respiration by KCN and SHAM observed in the 12-day-old green cotyledons and previously observed in older soybean leaves is not explained by characteristics of the mitochondria.

According to recent reviews (7, 12), the alternative respiratory pathway is widely distributed among various plant systems, and considerable efforts have been devoted to its study. However, questions still remain concerning some of the basic aspects of the path, including its activity *in vivo* and features governing its appearance in plant tissues. Among the complicating factors are the confounding effects of respiratory inhibitors in intact tissues of certain plants (2, 11, 14). De Visser and Blacquiere (2) reported a stimulatory effect of SHAM[3] at 5 mM and inhibition at 25 mM in roots of *Pisum sativum* and *Plantago* species. Spreen-Brouwer *et al.* (14) reported a similar response in intact roots of *Pisum* and maize (*Zea mays* L.) and suggested a solution for avoiding the stimulatory effect in estimating the alternative pathway. In recent studies with soybean leaf tissue (11), we reported on the age-dependent stimulation of O_2 uptake by both cyanide and SHAM when applied individually. We interpreted the stimulations by cyanide and SHAM as suggesting the presence of large alternative path capacities in these tissues. One major question is whether these unusual respiratory characteristics occur only in certain tissues of the soybean plant and whether they reflect mitochondrial activity.

Whereas there is some information concerning the nature of the respiratory pathways operating during imbibition and the earliest stages of soybean germination (8, 13, 18), there seem to be few reports of alternative path respiration in developing soybean seedlings. There is some evidence (16) that respiration of cotyledons from light-grown soybean seedlings is strongly cyanide resistant and may even be stimulated. Accordingly, we have examined the effects of KCN and SHAM on the respiration of cotyledons of developing soybean seedlings. Measurements included tissue and mitochondria respiration of both dark- and light-grown seedlings.

MATERIALS AND METHODS

Plant Material. Soybean (*Glycine max* [L.] Merr. cv Corsoy) seedlings were grown under both dark and light conditions. The seeds, surface-sterilized by soaking for 10 min in 5% NaOCl and washed in distilled H_2O, either were planted in moist vermiculite in vegetable crispers (25 × 19 × 9 cm rigid plastic boxes) or were rolled in moist germination paper (31 × 31 cm) and grown in the dark at 30 ± 2°C. The rolls were placed on end in a glass jar with 100 ml of distilled water in the bottom, with water added as required. The top of the jar was sealed with an inverted polyethylene bag. Seeds were also planted in 25-cm plastic pots containing a 1:1:1 mixture of soil, sand, and perlite and grown in a glasshouse under 14-h photoperiod.

Measurement of Cotyledon Respiration. Discs, 0.5 cm^2, were punched from the central region of cotyledons and rinsed in chilled, distilled H_2O. Cotyledons were pooled from five separate plants from each of three replications. For each separate measurement, five discs were used, and O_2 uptake was measured polarographically at 25°C by using a Clark-type O_2 electrode and conditions described previously (11). Cotyledon age is expressed in days, with 'day zero' being the day on which the seeds were planted.

[1] Journal Paper No. J-12882 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Projects No. 2496, 2724, and 2789. Supported in part by Pioneer Hi-Bred International, by U.S. Department of Agriculture CSRS Special grant 59-2191-1-2-010-0, and by U.S. Department of Agriculture Competitive Research grant 86-CRCR-1-2039.

[2] Permanent address: Department of Biological Sciences, Njala University College, University of Sierra Leone, P.M.B. Freetown, Sierra Leone, West Africa.

[3] Abbreviations: SHAM, salicylhydroxamic acid; FCCP, *p*-trifluoromethoxycarbonylcyanide phenylhydrazone.

Isolation of Mitochondria. Mitochondria were isolated from cotyledons of dark-grown seedlings as described by Day and Hanson (1) and as modified by Elthon *et al.* (4).

Cotyledons from light-grown seedlings were disrupted in a Polytron homogenizer (Kinematica) for 10 s at a setting of 7 in 2 volumes of ice-cold grinding medium (0.4 M mannitol, 1 mM EDTA, 50 mM Tris [pH 7.2], 1 mM $MgCl_2$, 8 mM cysteine, 5 mg/ml BSA, and 5% insoluble PVP). The homogenate was filtered through four layers of cheesecloth plus one layer of Miracloth and was centrifuged at 5,000*g* for 2 min. The lipid layer, when present, was carefully aspirated, and the supernatant was decanted and centrifuged again at 19,500*g* for 5 min. The resulting pellets were resuspended and combined in a total of 40 ml wash buffer (0.4 m mannitol, 10 mM Tris [pH 7.2], 5 mg/ml BSA). The suspension was centrifuged at 5,000*g* for 2 min. The resulting supernatant was removed and centrifuged at 19,500*g* for 5 min. The pellet was resuspended in 3 ml wash buffer and layered onto a discontinuous Percoll gradient. The Percoll gradient was prepared with final concentrations of 15, 27, and 55% Percoll, each containing 0.4 M mannitol, 10 mM Tris (pH 7.2), and 1 mg/ml BSA. Each gradient comprised 8 ml of 55%, 16 ml of 27%, and 12 ml of 15% Percoll. The gradient was centrifuged at 7,700*g* for 30 min. The material that accumulated at the interface of 27/55% Percoll was collected with a bent-tip Pasteur pipette, washed in 40 ml gradient wash buffer (0.4 M mannitol, 10 mM Tris [pH 7.2], and 1 mg/ml BSA) and sedimented at 9,500*g* for 15 min. The superatant was carefully aspirated. The pellet was resuspended in 40 ml gradient wash medium, and the centrifugation was repeated. The pellet of purified mitochondria was suspended in a final volume of 1.5 ml of the standard reaction medium consisting of 0.4 M mannitol, 10 mM Tris, 5 mM KH_2PO_4, and 1 mg/ml BSA (pH 7.2). Mitochondria isolated by this procedure were coupled and utilized NADH, succinate, or glycine as substrates.

Mitochondrial Respiration. Mitochondrial O_2 consumption was measured polarographically with a Clark O_2 electrode (Yellow Spring Instrument), and with NADH and succinate as substrates, at 25°C in 2 ml of reaction medium. Final substrate concentrations were 10 mM for succinate and 1 mM for NADH. When succinate was the respiratory substrate, mitochondria were pretreated with 85 M ATP. State 3 respiration was induced by addition of 200 nmol ADP. When inhibiors (KCN and/or SHAM) were used, they were added to mitochondria in state 3 after two cycles of ADP addition. FCCP was added during state 4 respiration after one cycle of ADP addition. KCN and SHAM were used at a final concentration of 2 mM, FCCP at 1 μM. KCN was dissolved in distilled H_2O and FCCP in 95% (v/v) ethanol/H_2O. SHAM was initially dissolved in just enough 95% ethanol and then brought to a final volume of 2 ml with distilled H_2O, and the pH was adjusted to 7.2 with KOH. Stock solutions were made fresh each time.

The electrode was calibrated against air-saturated water at 25°C, in which O_2 concentration was taken as 250 M. Each experiment was conducted three times, and duplicate assays performed each time.

Protein Determination. Protein was first precipitated by addition of 0.1 ml of 10% (w/v) trichloroacetic acid to 0.1 ml of the mitochondrial suspension. The pellet was redissolved in 0.2 ml of 0.4 N NaOH. The protein was then measured by the method of Lowry *et al.* (9) with BSA (fraction V) as standard.

RESULTS

Cotyledon Respiration. The effects of KCN and SHAM on the O_2 uptake rates of cotyledon discs from both dark- and light-grown seedlings, as a function of seedling age, are shown in Figure 1. Oxygen uptake rates of cotyledons from light-grown seedlings were higher than those of cotyledons from dark-grown seedlings during the early stages of development. After 9 d, however, the difference in the O_2 uptake rates decreased as a result of a decline of respiration in the light-grown seedlings.

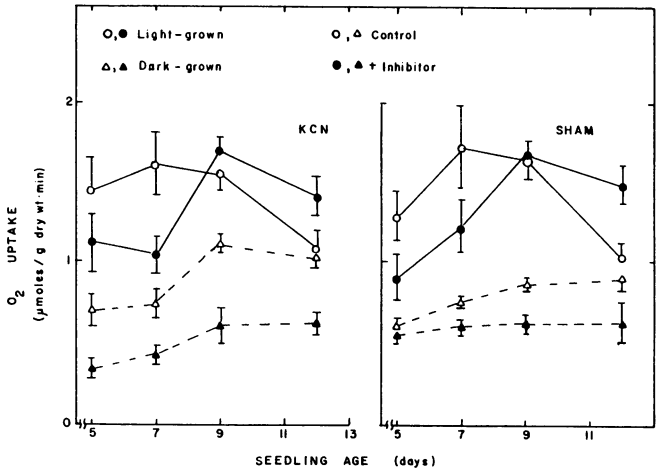

FIG. 1. Effect of KCN and SHAM on respiration of soybean cotyledon discs as a function of seedling age and growth conditions. Data points represent the mean of three independent determinations with a set of five seedlings each. O_2 uptake was measured polarographically at 25°C in 5.0 ml distilled water. Control rates are the rates before addition of inhibitors. Rates in the presence of inhibitors were taken after 30 min exposure to the inhibitor in a stirred, O_2-electrode cuvette, with the solution being aerated before the rate was measured. KCN and SHAM were used at a final concentration of 1 and 10 mM, respectively. Bars indicate SE.

The response to inhibitors of cotyledons from dark-grown seedlings was quite different from that of cotyledons from light-grown seedlings. Both showed a relatively high resistance to both KCN and SHAM when the inhibitors were applied individually. However, whereas O_2 uptake by cotyledons from dark-grown seedlings showed a somewhat greater and consistent level of sensitivity to both KCN and SHAM over the age period studied, the level of sensitivity in cotyledons from light-grown seedlings diminished with seedling age. Stimulation of as much as 30 to 40% by both inhibitors was observed after 9 d of growth. The response pattern observed with cotyledons from light-grown seedlings is similar to that reported by Tuquet and Dizengremel (16) for whole cotyledons of light-grown soybean seedlings. They did not detect cyanide stimulation until after 12 d of growth. In the presence of both KCN and SHAM, O_2 uptake was totally eliminated in both cotyledon types, except at ages 7 and 9 d when residual rates amounting to about 10% of control rates were observed (data not shown).

Mitochondrial Respiration. Figure 2 shows O_2 electrode traces depicting the effect of the sequential addition of ADP, KCN, and SHAM on coupled soybean cotyledon mitochondria. Inhibitors were used at a final concentration of 2 mM to ensure complete inhibition of the respective pathways. Mitochondria isolated from cotyledons of both dark- and light-grown soybean seedlings exhibited active cyanide-resistant oxidation of succinate and NADH. Glycine was also oxidized by mitochondria from light-grown seedlings, and this oxidation was also cyanide-resistant (data not shown). Although the differences in growth conditions may preclude direct comparison, it is perhaps worth noting that, for the most part, O_2 uptake rates of mitochondria from light-grown seedlings were considerably higher than those of mitochondria from dark-grown seedlings. This difference is in consonance with the respiration rates observed with the intact cotyledon tissues (Fig. 1).

The relative amounts of cyanide- and SHAM-sensitive respi-

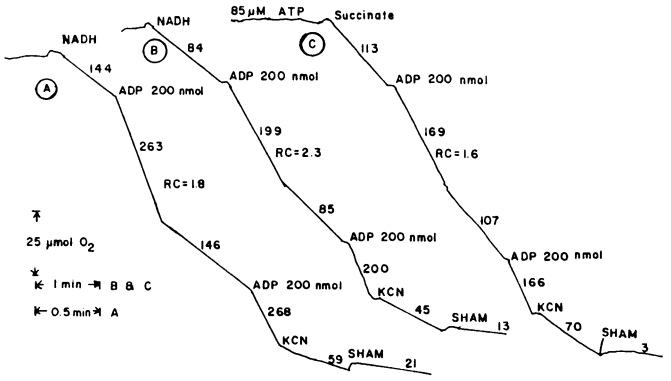

FIG. 2. Mitochondrial O_2 consumption traces. Traces are from different preparations of mitochondria isolated from cotyledons of 9-d-old light-grown (A) and dark-grown (B and C) seedlings. O_2 uptake assays were conducted at 25°C in 2.0 ml standard reaction medium at pH 7.2. Numerical values represent nmol O_2/min mg protein. State 3 to state 4 transitions have been sharpened to facilitate calculations of ADP/O. All other factors were as described in "Materials and Methods."

ration at various ages and with NADH and succinate as substrates are shown in Table I. Oxygen uptake rates by mitochondria from cotyledons of both light-grown and etiolated seedlings were equally strongly inhibited by KCN. Succinate oxidation was, however, less sensitive to inhibition by KCN. The greater cyanide-resistant respiration observed with succinate as substrate is consistent with the view that the ubiquinone pool associated with external NADH dehydrogenase and that associated with succinate dehydrogenase are not equally accessible to the alternative pathway. Succinate dehydrogenase may have a more specific association with the alternative oxidase (7, 10).

The age of light-grown cotyledons from which mitochondria were isolated did not seem to influence the degree of sensitivity to KCN. Inhibition by SHAM in the presence of KCN, the standard estimation of the alternative pathway capacity, remained unchanged over the period studied. Thus, the ratio of the two pathways remained constant. Cotyledon mitochondria from etiolated plants behaved differently. Sensitivity to KCN increased with seedling age, and sensitivity to SHAM in the presence of KCN decreased over time. In etiolated cotyledons, mitochondrial oxidation of NADH was increasingly dominated by the Cyt pathway as the relative contribution from the alternative pathway declined. In the presence of both KCN and SHAM, O_2 uptake during the entire growth period was inhibited by 92% or more.

Capacities of Cyt and Alternative Pathways. To ascertain whether the inhibitor responses observed with cotyledon discs reflected changes in the relative capacities of mitochondrial electron transport pathways, we measured the capacities of Cyt and alternative pathways for both light- and dark-grown seedlings and with NADH (Fig. 3) as substrate. O_2 uptake in mitochondria from all ages was coupled to phosphorylation. The average respiratory control ratio was 1.8. Throughout the growth period studied, the estimated Cyt capacity tended to be considerably larger than that of the alternative pathway for both NADH and succinate (data not shown) and for both dark- and light-grown seedlings. Total respiration (state 3) peaked on d 7, declining thereafter. This pattern was closely paralleled by the estimated Cyt capacity. In contrast, the estimated alternative path capacity tended to decline consistently with seedling age. Similar results (not shown) were observed with succinate as substrate.

Because the level of the electrochemical gradient present can influence the measurable Cyt chain capacity and may also have an influence on flow of electrons through the alternative pathway (3), the capacities of the Cyt and alternative pathways were measured in the presence of FCCP by using NADH as the substrate. The oxidation of exogenous NADH is well suited for investigating the effects of the electrochemical gradient because its oxidation is not dependent upon transport processes. FCCP was used to abolish the electrochemical proton gradient to allow maximum electron flux through the respiratory pathways. The addition of FCCP resulted in increased oxidation at all seedling ages studied (Fig. 4), indicating that the initial rate of NADH oxidation was limited by the magnitude of the electrochemical gradient (3). Thus, rates were more sensitive to cyanide in the presence of SHAM. In contrast, the rates were less sensitive to SHAM in the presence of cyanide, in both light- and dark-grown seedlings. Thus, when the initial rates of O_2 uptake are measured in the absence of FCCP, there is electron flow through both pathways. In the presence of FCCP, the initial rate is stimulated because the electrochemical gradient has been abolished. Under these conditions, the sensitivity to cyanide in the presence of SHAM is greater because electron flow through the Cyt pathway was not restricted by the electrochemical gradient before cyanide was added.

Our interpretation of the lesser sensitivity to SHAM in the presence of cyanide in the FCCP-treated cotyledons is that the uncoupling effect of FCCP caused diversion of electrons from the alternative pathway. When cyanide was added, electrons were not diverted back, thus less apparent SHAM inhibition was observed. However, the combined Cyt and alternative pathways did not exceed the rate in the presence of FCCP, indicating that the combined capacities did not exceed the NADH dehydrogenase capacity (4).

DISCUSSION

In recent studies from this laboratory (11), we have reported the stimulation of O_2 uptake by intact tissues of mature soybean

Table I. *Inhibition by KCN and SHAM in Mitochondria Isolated from Cotyledons of Light-Grown and Etiolated Soybean Seedlings*

Substrates were 1 mM NADH and 10 mM succinate. Inhibition of O_2 uptake is expressed as percentage of the state 3 rate after addition of 2 mM KCN or 2 mM SHAM. Values presented are means from three different preparations ± SE.

Inhibitor	O_2 Uptake (% of uninhibited state 3 rate)					
	5 d		9 d		12 d	
	NADH	*Succ*	*NADH*	*Succ*	*NADH*	*Succ*
			Light-Grown			
KCN alone	29 ± 3	59 ± 3	23 ± 3	48 ± 5	25 ± 5	
SHAM in presence of KCN	5 ± 1	4 ± 4	8 ± 2	25 ± 5	5 ± 3	
			Dark-Grown			
KCN alone	40 ± 2	50 ± 4	23 ± 3	40 ± 2	16 ± 1	62 ± 3
SHAM in presence of KCN	4 ± 2	3 ± 1	4 ± 3	3 ± 1	7 ± 1	3 ± 2

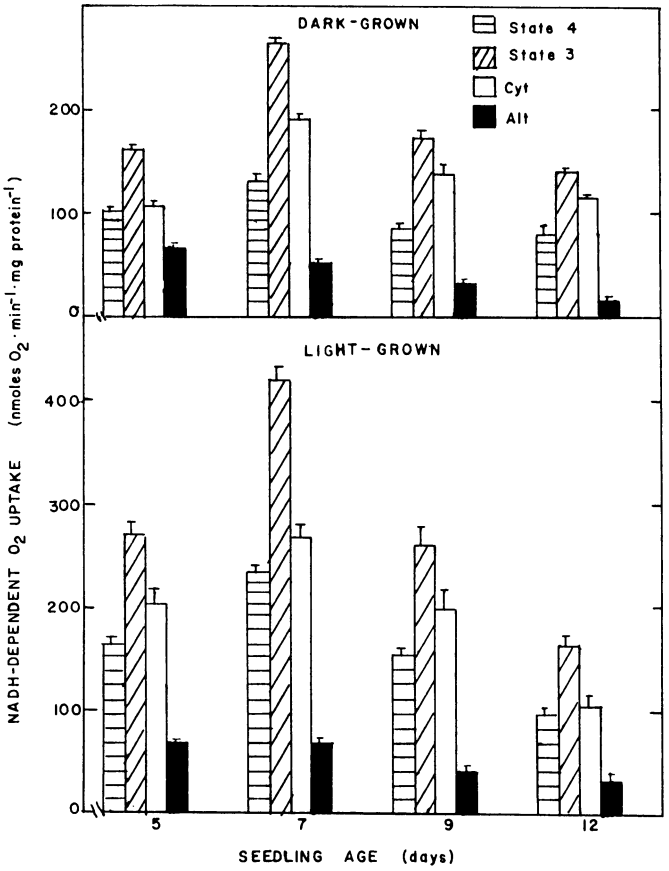

FIG. 3. Measurable capacities of the Cyt and alternative pathways in isolated soybean cotyledon mitochondria as a function of seedling age and with NADH as substrate. The Cyt capacity is the rate insensitive to 2 mM SHAM minus the residual rate after subsequent addition of 2 mM KCN. The capacity of the alternative path was estimated as the 2 mM KCN-insensitive rate minus the residual rate after the subsequent addition of SHAM. Data represent the mean of three experiments. State 3 rate was induced with addition of 200 nmol ADP. All other conditions were as described in "Materials and Methods." Rates are means from three different preparations ± SE.

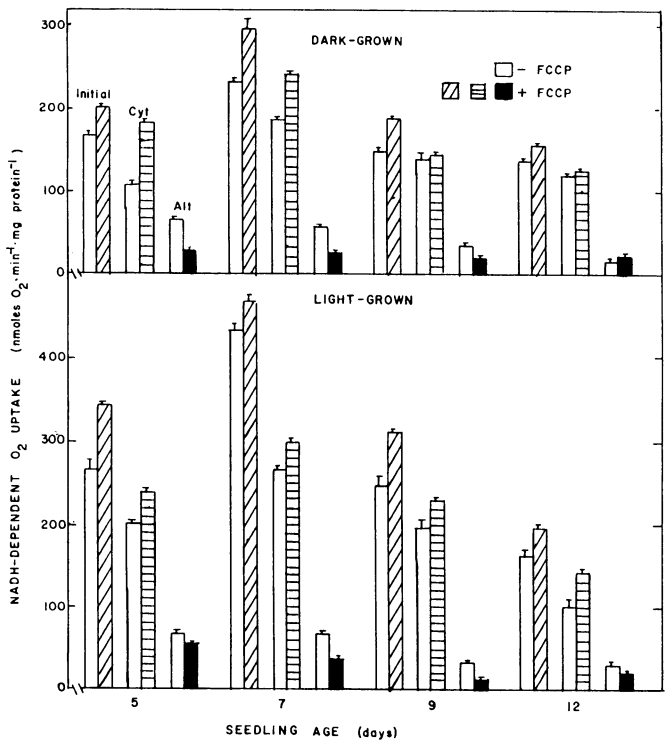

FIG. 4. Effect of the electrochemical gradient on measurable capacities of the Cyt and alternative paths in isolated soybean cotyledon mitochondria at different ages of light- and dark-grown seedlings. Assays were initiated upon addition of 1 mM NADH. The initial O_2 uptake rates in the presence of 1 μm FCCP are represented along with the capacities of the Cyt and alternative paths. The clear bars represent the corresponding rates in the absence of FCCP. Data represent the mean of three experiments. To allow for plotting of the data, all rates were normalized to the average initial rates (in the presence and absence of FCCP). Rates are from three different preparations ± SE.

leaves in the presence of either KCN or SHAM. We interpreted our results on the basis of the relative capacities and activities of the two mitochondrial electron transport pathways and suggested the presence of a potentially large SHAM-sensitive alternative path in soybean leaves. We now present further data indicating that the stimulation of O_2 uptake by KCN and SHAM is also observed in cotyledonary tissue from light-grown soybean seedlings beyond 9 d of growth. However, in combination, KCN and SHAM were inhibitory. KCN-stimulated O_2 uptake in cotyledons of light-grown soybean seedlings has been observed previously (16). This is the first report of SHAM-stimulated O_2 uptake in soybean cotyledonary tissue, although it has been observed in roots of pea (2) and maize plants (14) and in leaves of soybean plants (11). It is of interest that the inhibitor sensitivity pattern of O_2 uptake by cotyledonary tissue of light-grown seedlings differed markedly from that observed with cotyledonary tissue of etiolated seedlings. Oxygen uptake by etiolated cotyledonary tissue was cyanide- and SHAM-resistant throughout the growth period studied, but in no case was O_2 uptake stimulated by either KCN or SHAM. The 40 to 60% and 10 to 30% inhibition of O_2 uptake in etiolated cotyledonary tissue by KCN and SHAM, respectively, is quite typical of intact tissues (6). It would seem that the stimulations of O_2 uptake in the soybean plant by respiratory inhibitors are observed only with green tissues. Tetley and Thimann (15), in considering the contrast between the cyanide inhibition of the respiration of etiolated oat (*Avena sativa* L.) leaves and the cyanide promotion of the respiration of green leaves, reached a similar conclusion and suggested an association between the KCN-stimulated system and the development of the photosynthetic apparatus. Perhaps there is a similar association between the mechanism responsible for SHAM stimulation and the development of the photosynthetic apparatus.

The stimulation of O_2 uptake by cyanide has been interpreted as a result of decreased ATP production which in turn stimulates carbohydrate catabolism. The electrons produced are transferred to O_2 via the alternative pathway (5). If this interpretation is correct, then the unused capacity of the alternative pathway must exceed the activity of the Cyt pathway. Our measurements of the capacity of the alternative pathway in green cotyledons indicate rather low capacities of the alternative pathway and the capacities decline with age. The capacity was lowest when cyanide stimulation was greatest. These results do not seem consistent with the mechanism of cyanide stimulation stated above.

The stimulation of O_2 uptake by SHAM is very difficult to interpret because of the known side effects of SHAM. One such effect is the stimulation of a peroxidase (2, 14, 17). This peroxidase has been suggested as possibly responsible for SHAM stimulation (14). However, in potato callus tissue, hydroxamates stimulate O_2 uptake only in the presence of exogenous NADH. Thus, this peroxidase does not appear to function in O_2 uptake in potato callus (17). Soybean leaves contain this SHAM stimulated peroxidase (M Miller, unpublished data) but no role in O_2 uptake in the absence of SHAM can yet be assigned to it. We

have only observed SHAM stimulation in green tissue whereas the peroxidase has been measured in several nongreen tissues (14, 17).

Although there are reports in the literature of cyanide stimulation of respiration and of SHAM stimulation of respiration, we are not aware of other tissues where stimulation by both compounds is observed. Based on previously suggested explanations of these stimulations, it seems unlikely the two are linked. It remains to be determined what it is about green soybean tissues that differ from other photosynthetic tissues in which O_2 uptake is not stimulated by either cyanide or SHAM or both.

LITERATURE CITED

1. DAY DA, JB HANSON 1977 On methods for the isolation of mitochondria from etiolated corn shoots. Plant Sci Lett 11: 99–104
2. DE VISSER R, T BLACQUIERE 1984 Inhibition and stimulation of root respiration in *Pisum* and *Plantago* by hydroxamate. Its consequences for the assessment of alternative path activity. Plant Physiol 75: 813–817
3. ELTHON TE, CR STEWART 1983 A chemiosmotic model for plant mitochondria. BioScience 33: 687–692
4. ELTHON TE, CR STEWART, CA MC COY, WD BONNER JR 1986 Alternative respiratory path capacity in plant mitochondria: effect of growth temperature, the electrochemical gradient and assay pH. Plant Physiol 80: 378–383
5. LAMBERS H 1985 Respiration in intact plants and tissues: its regulation and dependence on environmental factors, metabolism and invaded organisms. *In* R Douce, DA Day, eds, Encyclopedia of Plant Physiology, Vol 18, Higher Plant Cell Respiration. Springer-Verlag, Berlin, pp 418–473
6. LAMBERS H, DA DAY, J AZCON-BIETO 1983 Cyanide-resistant respiration in roots and leaves. Measurements with intact tissues and isolated mitochondria. Physiol Plant 58: 148–154
7. LANCE C, M CHAUVEAU, P DIZENGREMEL 1985 The cyanide-resistant pathway of plant mitochondria. *In* R Douce, DA Day, eds, Encyclopedia of Plant Physiology, Vol 18, Higher Plant Cell Respiration. Springer-Verlag, Berlin, pp 202–247
8. LEOPOLD AC, ME MUSGRAVE 1980 Respiratory pathways in aged soybean seeds. Physiol Plant 49: 49–54
9. LOWRY OH, NJ ROSEBROUGH, AL FARR, RJ RANDALL 1951 Protein measurements with the Folin phenol reagent. J Biol Chem 193: 265–275
10. MOORE AL, PR RICH 1985 Organization of the respiratory chain and oxidative phosphorylation. *In* R Douce, DA Day, eds, Encyclopedia of Plant Physiology, Vol 18, Higher Plant Cell Respiration. Springer-Verlag, Berlin, pp 134–172
11. SESAY A, CR STEWART, RM SHIBLES 1986 Effects of KCN and salicylhydroxamic acid on respiration of soybean leaves at different ages. Plant Physiol 82: 443–447
12. SIEDOW JN 1982 The nature of the cyanide-resistant pathway in plant mitochondria. *In* LL Creasy, G Hoardena, eds, Recent Advances in Phytochemistry. Plenum Press, New York, pp 47–83
13. SIEDOW JN, ME GIRVIN 1980 Alternative respiratory pathway. Its role in seed respiration and its inhibition by propyl gallate. Plant Physiol 65: 669–674
14. SPREEN-BROUWER K, T VAN VALEN, DA DAY, H LAMBERS 1986 Hydroxamate-stimulated O_2 uptake in roots of *Pisum Sativum* and *Zea mays*, mediated by a peroxidase. Its consequences for respiration measurements. Plant Physiol 82: 236–240
15. TETLEY RM, KV THIMANN 1974 The metabolism of oat leaves during senescence. Plant Physiol 54: 294–303
16. TUQUET C, P DIZENGREMEL 1984 Changes in respiratory process in soybean cotyledons during development and senescence. Z Pflanzenphysiol 114: 355–359
17. VANDER PLAS LHW, H GUDE, MJ WAGNER 1987 Hydroxamatic-activated peroxidases in potato tuber callus. Interaction with the determination of the cytochrome and alternative pathways. Physiol Plant 70: 35–45
18. YENTUR S, AC LEOPOLD 1976 Respiratory transition during seed germination. Plant Physiol 57: 274–276