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Temperature and Oxygen Effects on ^{14}C-Photosynthate Unloading and Accumulation in Developing Soybean Seeds

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ABSTRACT

The environmental sensitivity of the processes associated with the import of photosynthate by developing soybean seeds was investigated within intact fruit and with excised, immature embryos. Intact pods of field-grown (*Glycine max* [L.] Merr.) Amsoy 71 soybeans were subjected to localized regimes of 0, 21, or 100% O_2 and 15, 25, or 35°C during pulse-chase translocation experiments and, 2.5 hours later, the uptake and distribution of ^{14}C-photosynthate among dissected fruit tissues determined. In other experiments, excised embryos were incubated in [^{14}C]sucrose solutions under various experimental conditions to separate the effects of these treatments on accumulation by the embryos from those which may operate on phloem unloading in the maternal seedcoat.

Import of ^{14}C-photosynthate by intact soybean fruit was both temperature- and O_2-dependent. This dependency was shown to occur only within the seeds; import by the pod walls was essentially insensitive to fruit temperature or O_2 treatments. The embryos of anaerobic fruit were completely unlabeled, regardless of fruit temperature. But under anaerobic *in vitro* incubation conditions, uptake of [^{14}C]sucrose in excised embryos was only 30% less than that in aerobic *in vitro* conditions. The data suggest that, within intact fruit, anoxia prevented sucrose efflux from the seed coat phloem and any subsequent uptake by the embryo. The demonstrated energy dependence of phloem unloading may reflect requirements for membrane integrity or energy metabolism in the companion cell-sieve element complex, consistent with a facilitated unloading process.

Collectively, these data characterize the environmental sensitivity of photosynthate import in developing soybean fruit. They imply that environmental regulation of import may occur at both the embryo level and at the phloem terminals within the seed coat.

Temperature extremes during critical growth stages are associated with many of the observed seasonal fluctuations among components of agronomic productivity in soybean: seed number, rate and duration of seed growth, final seed size, and nutritional quality (4, 5, 9, 10, 23). Regulation of seed growth is, to some extent, mediated through effects of temperature on photosynthate uptake or utilization by the developing reproductive tissues. Recently, Egli and Wardlaw (5) reported that temperatures above 30/25°C (day/night) enhanced embryo growth rate when cultured *in vitro*, but reduced the seed size of intact fruit. Growth rates of these seeds were unaltered by a shift to high temperatures during seed formation, but duration of growth decreased. Conversely, temperatures of 18/13°C reduced the growth rates of seeds *in situ* and of embryos cultured *in vitro*. They concluded that the overall effect of temperature was directly on the seed itself, instead of on the ability of the maternal plant to supply photosynthate to developing seeds (5). Inasmuch as a portion of the seed through which photosynthate passes is maternal tissue, a further distinction is necessary. Indeed, work with *Pisum* (14, 29) demonstrated that the movement of tracers into young fruit, especially seeds, is temperature-sensitive. Presently, little is known of the sites or mechanisms of the temperature dependence observed by these workers. However, recent studies of the processes associated with photosynthate import suggest likely locations.

Photosynthate imported from the pod must pass through the maternal seed coat before becoming available for absorption by the embryo (25). However, little is known of the mechanisms or environmental sensitivity of the processes which control photosynthate release to the embryo from the seed coat. More is known of the subsequent cellular events associated with the absorption of photosynthate by the cotyledons from the free space between the embryo and surrounding maternal tissues. An important component of this absorption is known to involve a carrier-mediated transport system (12, 13), but its environmental sensitivity is unknown. Although it is apparent that the rate of photosynthate release from the seed coat will determine the rate of absorption by the embryo *in situ*, possible effects of absorption by the embryo on efflux from the seed coat can easily be envisioned.

In the few studies that have focused on transport events in legume seeds (8, 14, 15, 25, 29), little has been learned about the role that environmental or physiological controls of transport play in determining agronomic productivity. To effect regulation of photosynthate distribution and seed growth, further information is needed about the limitations of the processes associated with sucrose uptake and utilization. The following report is an evaluation of the environmental sensitivity of ^{14}C-photosynthate uptake by both intact developing seeds and isolated soybean embryos.

MATERIALS AND METHODS

Intact Plant Studies.

Pod Pretreatment. Within a population of field-grown (24) soybeans, 3-m lengths of three adjacent rows were thinned to nine plants each. Plants were selected on the basis of uniformity at nodes 10 to 14 of the plant mainstem using a previously reported procedure (25), with the following modifications: as mentioned, plants were in adjacent rows, undesired plants were removed at the soil level, lateral branches were removed to open the canopy further, and any undesired pods were removed from the experimental node. Installed near each plant at the height of the experimental node was a waterjacketed acrylic pod chamber.

The following morning, all pods at the experimental nodes were sealed into the pod chambers which had been pre-equilibrated at either 15, 25, or 35°C, and through the interior of which passed either 0, 21, or 100% O_2. Any air gaps were sealed with soft putty.

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At each plant within a row was a pod chamber assigned one of the nine temperature *versus* O_2 combinations. All experimental pod regimes were then maintained during a 4-h pretreatment period following insertion of the last pods into the chambers.

Chambers were supplied with water and gas under pressure by thick-walled flexible tubing from three controlled temperature water baths and compressed gas cylinders, respectively. Water was recirculated to the water baths but gases were vented via tubing to the atmosphere 2 m from the plants. Pod chamber temperatures were maintained to ±1°C as determined by copper-constantan thermocouples. Chambers were connected in parallel and partially insulated to minimize temperature differences. Light reaching the pods was reduced but not eliminated by the insulation.

$^{14}CO_2$ Labeling. Following the 4-h pretreatment period, large assimilation chambers (90 × 120 × 300 cm) constructed of IR-transparent polypropylene ("Propafilm C110," Imperial Chemicals Industries [ICI Americas], Wilmington, DE) were placed over the rows. Two 12-cm Muffin fans (Rotron Inc, Woodstock, NY) within each chamber provided rapid air circulation. Into each large chamber, 20 μCi $^{14}CO_2$ were introduced from a small pressurized cylinder and the fans circulated the air rapidly during a 20-min period of photosynthetic labeling. Although the labeled gas was supplied at 300 μl l^{-1} CO_2 (1.1% O_2), no attempt was made to determine the ambient CO_2 concentration or photosynthetic rates during the labeling period. Leaf temperatures within the assimilation chambers were 3 ± 1°C above those outside. The large assimilation chambers were then removed and the 27 plants allowed a 2.5-h "chase" period of translocation. This interval was selected on the basis of previous kinetic data (25) to be approximately 2 h beyond the time of arrival of the ^{14}C-photosynthate in seeds of pods exposed to similar ambient mid-day conditions but without pod treatments. In those pods, approximately 40% total label in the fruit by 2.5 h had passed through the seed coats to the embryos. The translocation interval chosen in the present experiment was expected to permit maximum expression of environmentally induced deviations from this "normal" distribution of label. Pod treatment regimes were maintained for a total of about 7 h (4-h pretreatment + 20-min label + 2.5-h chase) on a mostly sunny day in late August when the pods at the experimental nodes were between 40 and 50 days of age.

Recovery and Analysis of ^{14}C-Photosynthate. After the 2.5-h translocation period, treated pods were removed and quickly frozen on solid CO_2. Frozen fruit were later dissected into pod walls (including major vascular bundles), seed coats, and cotyledons (including embryonic axes). The frozen cotyledons were briefly rinsed with ice-cold H_2O (2–3 s) to remove the embryo sac tissue. It appeared that only the embryo sacs and perhaps the epidermal cotyledonary cells may have thawed during this operation, for a rinse fraction containing the embryo sac tissue was found to possess only slightly higher levels of radioactivity than embryo sac tissue removed from frozen embryos without rinsing. All tissues were extracted with 80% ethanol (v/v) in a microsoxhlet apparatus; cotyledons for 36 h, pod walls and seed coats for 24 h. Ethanol-soluble photosynthate was dried at 40°C under reduced pressure. Samples from pod walls and cotyledons were resuspended in 2.0 ml 50% ethanol (v/v) and the radioactivity in aliquots determined by liquid scintillation spectroscopy in Aquasol II cocktail (New England Nuclear) using external standard quench correction.

Dried seed coat extracts were redissolved in a small amount of 50% ethanol (v/v), and the neutral sugars eluted with water through columns containing Dowex 50 W cation exchange resin layered over Dowex 1 anion exchange resin separated by a narrow band of Sephadex G-25. The specific radioactivity of the [^{14}C]sucrose component of the eluant was determined by colorimetry (24) and by liquid scintillation spectroscopy.

Radioactivity in the ethanol-insoluble fraction was determined by liquid scintillation spectroscopy following combustion with a Harvey OX-300 biological oxidizer (R. J. Harvey Instrument Corp., Hillsdale, NJ).

***In Vitro* Uptake Studies.**

Plant Culture. Nodulated plants of soybean (*Glycine max* (L.) Merr. 'Amsoy 71') were grown in a controlled environment room in which illumination (1.0×10^3 μe $m^{-2}s^{-1}$ PAR) was provided by a mixture of incandescent and Sylvania GRO-LUX wide spectrum fluorescent lamps. Principal growth conditions (30/17 °C, 14-h photoperiod) were interrupted from 45 to 60 days after planting to induce flowering (30/17 °C, 9-h photoperiod). Flowers were tagged to provide a population of 40- to 50-day-old fruit.

Embryo Isolation. For experiments in which no pretreatment of the intact pods was involved, pods were routinely harvested at 0800 to 0900 daily. Seeds were removed from the pod walls and then given a 5-min soak at room temperature in buffer (5 mM Mes, pH 6.0) to facilitate subsequent seed coat removal. Excised but undamaged embryos (comprised of the embryonic axis plus attached cotyledons) were washed in 5 mM Mes (pH 6.0) for 20 to 25 min at room temperature unless a specific temperature treatment was to be imposed during [^{14}C]sucrose incubation, in which case the buffer wash was also at that temperature. The buffer wash solutions were generally equilibrated with air, unless a specific O_2 treatment was to be imposed during [^{14}C]sucrose incubation, in which case the buffer wash was carefully equilibrated with the appropriate gas.

For *in vitro* experiments which involved pretreatment of the intact pods with various gases, somewhat different isolation procedures were employed. Selected pods were exposed at room temperature to 0, 21, or 100% O_2 using the procedures of the field studies. Pretreatment starting times were staggered to allow sequential harvesting of treated pods. After 4 h, pods were individually harvested and the seeds quickly excised and placed in room temperature buffer (5 mM Mes [pH 6.0], maintained in equilibrium with appropriate gas) for 5 to 7 min. Only 3 to 5 s were required to remove the seeds of each pod and immerse them in buffer. Later, during seed coat removal, a stream of the appropriate gas directed at the dissection area minimized exposure to ambient O_2. Seeds were individually removed from the buffer and their seed coats removed, resulting in a 10- to 15-s exposure to ambient conditions. Following this rapid excision, embryos were placed in preincubation buffer (room temperature 5 mM Mes [pH 6.0], equilibrated with the appropriate gas) for 20 to 25 min.

Uptake Experiments—Embryos were incubated in triplicate in medium (three embryos/15 ml) containing 5 mM Mes (pH 6.0) and diluted [^{14}C]sucrose (1 mCi/mmol) in a background of unlabeled sucrose of varied concentrations, as detailed in the figure and table legends. Uptake was from solutions in equilibrium with gases from cylinders containing 0, 21, or 100% O_2 (balance N_2). As with the preincubation wash buffer, solutions were carefully equilibrated with appropriate gases 1 h prior to incubation. Before N_2 equilibration, appropriate solutions were first degassed in an ultrasonic bath under reduced pressure. Incubation temperature was maintained at 15, 25, or 35°C by a shaking water bath.

Uptake experiments were initiated by rapidly transferring embryos from the buffer solutions to appropriately equilibrated incubation flasks. Less than 5 s were typically necessary to blot the embryos and drop them into the appropriate vial. A stream of the appropriate gas was directed at each embryo as it was blotted and dropped into the ^{14}C solutions. Bubbling of the gases through the incubation medium (about 150 ml/min) provided constant agitation and maintained gas equilibrium. Plastic snap caps fitted with two blunt hypodermic needles provided inlet and outlet ports for gas flushing. The O_2 content of the gases exiting the flasks was determined periodically with an O_2-analyzer (model 209, Westinghouse Electric Corp., Pittsburgh, PA). The incentive for the numerous precautions employed to minimize exposure to ambient

O_2 was the recent finding (18) that a 1-min exposure of red beet storage tissue to air after anaerobiosis is sufficient to restore normal ATP levels and ion influx.

Sucrose uptake was terminated by sequentially collecting the embryos by Millipore filtration. After a 2-s rinse with ice-cold buffer, they were washed for 10 min in a large volume of ice-cold Mes (5 mM, pH 6.0) to remove labeled sucrose from the free space (as determined in preliminary efflux studies). Buffer wash solutions were agitated and maintained in equilibrium with appropriate gases throughout the wash period. Embryos were then blotted dry, quickly frozen on solid CO_2, and the accumulated radioactivity determined by liquid scintillation spectroscopy following tissue combustion with a Harvey OX-300 biological oxidizer (R. J. Harvey Instrument Corp., Hillsdale, NJ). Rates of sucrose uptake were calculated from the initial specific activity of the incubation media and are expressed as μmol sucrose uptake · h^{-1} · 100 mg fresh (frozen) $weight^{-1}$.

RESULTS AND DISCUSSION

Sucrose Import Route. Recent anatomical and physiological studies (25, 26) of the fruit tissues involved in photosynthate import have demonstrated that translocation of sucrose to the developing embryonic cotyledons occurs from the phloem of the mother plant through the vascular bundles of the pod, which in turn enter the seeds via the funiculus. Upon entering the seeds, photosynthate is rapidly and evenly distributed throughout the seed coat within an anastamosing network of minor veins (25, 26). Each of these consist of 2 to 10 small sieve tubes with large accompanying companion cells enclosed in a parenchyma sheath (26). No xylem is present. Literally no information is available on the nature of the transport mechanism facilitating sucrose exit from the sieve tubes; however, the symplastic linkage of the phloem and companion cells provides circumstantial evidence for a direct or indirect metabolic component (26).

Following unloading, sucrose moves intercellularly across about 100 μm aerenchyma to the free space separating the seed coat and the embryo. This movement may be by diffusion as it separates by about 1 h sucrose unloading and subsequent uptake by the embryo (25). The accumulation by the embryo of sucrose released from the seed coat appears to include both passive and active components. Lichtner and Spanswick (13) have provided evidence that the active uptake of exogenous sucrose is by a sucrose-proton co-transport system in the plasmalemma of cotyledon cells. Passive uptake of exogenous sucrose apparently increases linearly with concentration, maintaining substantial rates of accumulation in the presence of inhibitors of the active component (13). This aspect of assimilate accumulation is exploited in the present experiments to separate unloading from the subsequent uptake by the embryo. The lack of symplastic connections between the embryo and the seed coat *in situ* allowed the easy removal of intact embryos from the maternal tissues for the *in vitro* uptake studies.

Characteristics of Uptake by Intact Pods. During the 2.5-h translocation period following labeling of the plants with $^{14}CO_2$, very little ^{14}C-photosynthate was imported by developing fruit maintained at 15°C, regardless of ambient O_2 level (Fig. 1a). Higher pod temperatures significantly enhanced the uptake of label by aerobic fruit, especially those exposed to 100% O_2 during the pulse-chase experiment. Conversely, very little ^{14}C-photosynthate was imported by anaerobic fruit, regardless of temperature. Localized subambient O_2 treatments have been shown to inhibit energy metabolism and reproductive growth of soybeans and other crop species (20). In a series of published abstracts, Quebedeaux and co-workers (17, 21, 22) suggested that a 5% O_2 atmosphere surrounding soybean fruit inhibits seed energy metabolism and thus reduces photosynthate import. It seems that, by reducing the external O_2 level to 5%, these authors created near

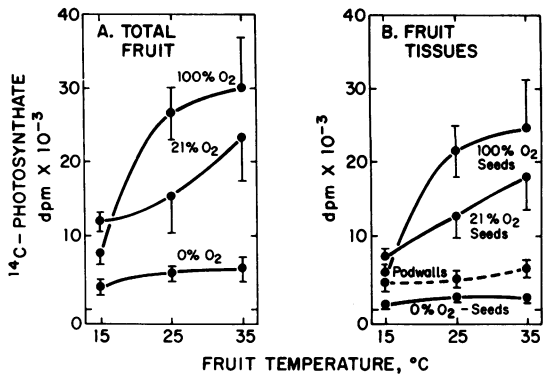

FIG. 1. Effects of fruit temperature and O_2 treatments on translocation of ^{14}C-photosynthate into soybean fruit (A), and its partitioning between pod walls and seeds 2.5 h after application of $^{14}CO_2$ (B). Pod walls include the radioactivity in major pod bundles and represent all treatments since none were significantly different. Entries represent the mean radioactivity ± SD of nine fruit (27 for pod wall entries) labeled and harvested as described in the text.

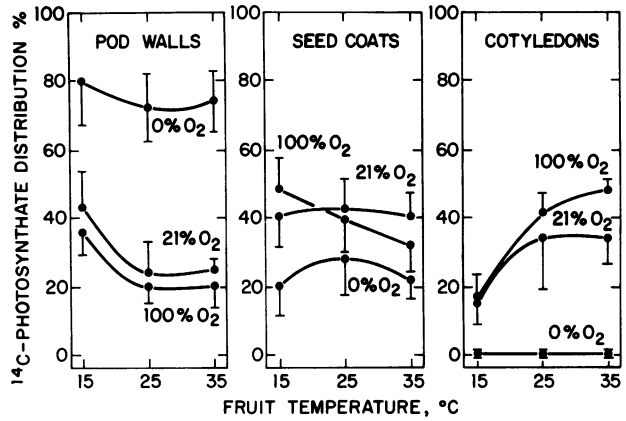

FIG. 2. Relative distribution among tissues of ^{14}C-photosynthate imported by fruit exposed to treatments of 15, 25, 35° C and 0, 21, 100% O_2. Each value is the mean distribution ± SD of appropriate tissues of nine fruit.

anaerobic internal conditions (6). They speculated that phloem unloading and biosynthesis of secondary products might be involved in the observed inhibition of seed growth.

The present experiments show that temperature and O_2 treatments influenced photosynthate uptake by affecting processes only within the seed (Fig. 1b). The pod walls were only a minor sink for incoming photosynthate and were unaffected by temperature or O_2 treatments. That the pod wall pericarp of Amsoy 71 fruit is only a minor photosynthate sink, despite extensive vascularization, has been previously noted (24, 25). Almost no label could be recovered from seeds of anaerobic pods, regardless of fruit temperature. With 21% O_2 surrounding the fruit, however, approximately 2-fold more ^{14}C-photosynthate was taken up by the seeds in the 2.5-h period after $^{14}CO_2$ labeling; the level increasing linearly with temperature from 15 to 35°C. Photosynthate uptake by seeds was enhanced even more over 15°C when the pods were exposed to 100% O_2 and 25 or 35°C (4-fold increase).

Distribution of Label within Anaerobic Fruit. To investigate the specific sites of environmental regulation, the relative distribution of ^{14}C-photosynthate among dissected fruit tissues was determined (Fig. 2). Under anaerobic conditions, all of the label was retained in maternal tissues: approximately 75% in the pod walls and 25% in the seed coats. No label was detected in the embryonic cotyledons of these fruit, regardless of the fruit temperature (Fig. 2). Anaerobiosis undoubtedly reduced the embryonic capacity for

Table I. *Influence of Anaerobiosis on Uptake of Exogenous Sucrose by Excised Soybean Embryos*

Incubation media also contained 5 mM Mes (pH 6.0). Incubation was for 1 h at 35°C, followed by a 10-min wash with ice-cold buffer. Gases were bubbled throughout the experiments. Each value represents the mean plus SD (in parentheses) of 12 embryos.

Concentration	Sucrose Uptake Rate in O_2 Regime		Inhibition Due to Anaerobiosis
	100%	0%	
mM	*μmol·h⁻¹·100 mg fresh wt⁻¹*		%
1.0 (50 nCi/μmol)	0.045 (0.004)	0.035 (0.004)	23.3%
10.0 (20 nCi/μmol)	0.34 (0.07)	0.25 (0.06)	26.5%
100.0 (10 nCi/μmol)	1.18 (0.12)	0.80 (0.17)	32.2%

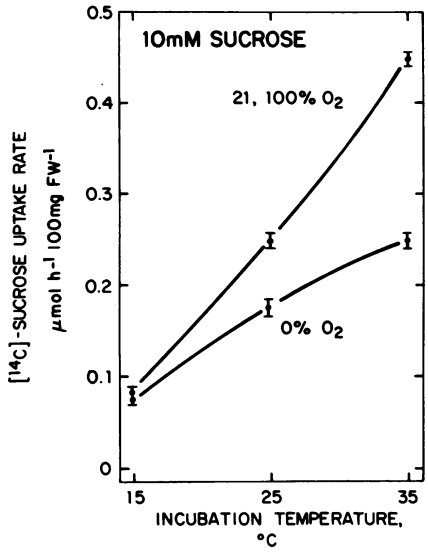

FIG. 3. Rates of [^{14}C]sucrose uptake by excised immature soybean embryos from 10 mM buffered solutions (5 mM MeS, pH 6.0) maintained in equilibrium with 0, 21, or 100% O_2 the mean ± SD of nine embryos. Data for 21 and 100% O_2 were indistinguishable.

Table II. *Uptake of Exogenous Sucrose by Embryos Isolated from Pods Pretreated with O_2*

Pretreatment[a] and Incubation[b] Regime	Sucrose Uptake Rate[c]	Inhibition (Relative to 100% O_2 Treatment)
% O_2	*μmol·h⁻¹·100 mg fresh wt⁻¹*	%
0	0.033 (0.004)	29.8
21	0.048 (0.002)	0
100	0.047 (0.003)	

[a] Intact pods exposed to appropriate gases within plastic chambers for 4 h at room temperature. Gas flow rate was approximately 0.5 L min^{-1}.

[b] Incubation for 1 h at 35°C in 1 mM sucrose (50 nCi ^{14}C/μmol) in 5 mM Mes (pH 6.0). All solutions were in equilibrium with 0, 21, or 100% O_2 (balance N_2) as detailed in the text.

[c] Values represent the means and standard deviations (in parentheses) of 9 embryos.

sucrose uptake, perhaps by eliminating or lessening the active component identified recently by Lichtner and Spanswick (13). However, their work predicts that the diffusion component of sucrose uptake, probably unaffected by anoxia, extrapolates linearly to 0 mM sucrose. Thus, in the present experiment, only if the cotyledons had not been presented ^{14}C-photosynthate by the seed coats would they be totally unlabeled. This suggests that, in anaerobic soybean fruit, ^{14}C-photosynthate import was prevented by the inhibition of phloem unloading and sucrose efflux from the seed coat.

To test this hypothesis, isolated embryos were incubated under aerobic and anaerobic conditions in a range of sucrose concentrations likely to include those normally encountered by developing soybean embryos *in situ*. Over a 100-fold range of [^{14}C]sucrose concentrations, uptake by isolated embryos under anaerobic conditions was fully 68 to 77% of that under aerobic conditions (Table I). Accumulation from sucrose solutions in equilibrium with both 21 and 100% O_2 was equivalent. Uptake of [^{14}C]sucrose by isolated embryos was temperature-dependent, regardless of O_2 availability (Fig. 3). At all temperatures, accumulation rates were equivalent in 21% and 100% O_2 treatments. In other experiments (Table II), embryos were isolated from pods which had been pretreated for 4 h with either 0, 21, or 100% O_2 (balance N_2). As detailed under "Materials and Methods," efforts were made during embryo isolation and [^{14}C]sucrose incubation to retain the pretreatment experience with respect to O_2 availability and, it was hoped, tissue ATP levels. As before, anaerobic embryos were able to accumulate sucrose at a rate which was about 70% of that of aerobic embryos (Table II). This uptake by excised anaerobic embryos contrasts with that of unexcised anaerobic embryos (Fig. 2), consistent with the conclusion that efflux from the seed coat had been prevented under anaerobiosis.

Recent studies by Wolswinkel (30) on *Vicia faba* stem tissue parasitized by *Cuscuta* (dodder) have indicated that sugar efflux from the sieve tube is dependent on metabolism, as evidenced by its inhibition by low temperature, dinitrophenol, and azide. This seems to be the only published evidence of energy-dependent phloem unloading; however, few studies have addressed this question (27).

Further evidence that anaerobiosis prevented phloem unloading was obtained upon examination of the seed coat sucrose pool. Under anaerobic conditions, the seed coat sucrose pool was almost entirely unlabeled (Fig. 4), suggesting that the exit of sucrose from the phloem is energy-dependent. It is not clear, however, that the restriction of label to the seed coat is due solely to decreased energy metabolism in the cells responsible for unloading (*e.g.* companion cells), for anaerobic treatments are known to induce confounding side effects, such as leaky membranes and resultant altered cell turgor (7). But, had the membranes at the seed coat unloading sites become leaky, the seed coat sucrose pool would have been more heavily labeled, the embryos surely would have been labeled, and a greater import of ^{14}C-photosynthate would have occurred in the anaerobic fruit than was observed (Figs. 1,

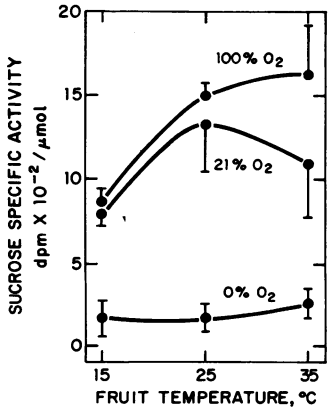

FIG. 4. Specific activity of extractable sucrose of seed coats isolated from fruit experiencing 15, 25, 35°C and 0, 21, 100% O_2. Each value represents the mean ± SD of seed coats from nine fruit analyzed as described in the text.

2, 4).

While literally nothing is known of the phloem-unloading mechanism, it is tempting to speculate that unloading occurs via the sieve element-companion cell complex down a concentration gradient from the phloem to the apoplast of surrounding seed coat tissue, facilitated by carrier-mediated passage of the plasma membrane. Although direct evidence for mediated transport during unloading is lacking, it is difficult otherwise to envision rapid, controlled efflux from the phloem, given the relative impermeability of plant membranes to sucrose and the resistance of the phloem to leakage (27). The demonstrated sensitivity of unloading to anoxia suggests an energy requirement for membrane integrity and/or transport which may influence sucrose import under certain conditions. It does not seem possible to resolve further the unloading mechanism on the basis of these data. Additional studies of the mechanisms of phloem unloading are needed, particularly with respect to those occurring within reproductive sinks.

Import under Aerobic Conditions. Increasing the O_2 level in the atmosphere surrounding the fruit to 21 or 100% resulted in an extensive flux of photosynthate through the seed coats to the embryos (Fig. 2) during the 2.5-h period following the $^{14}CO_2$ application. A marked temperature dependency was apparent. At 15°C, more than 80% label remained in maternal pod wall and seed coat tissues. Only a small percentage could be recovered from the embryos, even from those fruit exposed to 100% O_2 (Fig. 2). Since the sucrose specific activity of the seed coat was similarly affected at 15°C, it seems that unloading as well as uptake by the embryo may have been affected.

Higher temperatures resulted in about a 2-fold greater flux of label from the maternal tissues to the embryo. When the temperature of aerobic fruit was increased from 15 to 25°C, neither the relative amount of label (Fig. 2) nor total sucrose in the seed coats (data not shown) increased. However, between 15 and 25°C, the sucrose specific activity in the seed coat increased nearly 2-fold (Fig. 4), indicating an enhanced rate of turnover of the sucrose pool. That this enhanced unloading at higher temperature may be in direct response to more rapid accumulation by the embryos is suggested by the pronounced temperature dependence of [^{14}C]-sucrose uptake by excised (Fig. 3) and unexcised (Fig. 2) embryos. Presently, I cannot make a definitive cause-effect assignment for regulation of photosynthate import to either maternal (seed coat) or embryonic tissues. Perhaps the developing embryo is capable of assuming primary control over maternal unloading sites in the seed coat through enhancement of their membrane permeability as in the case of *Cuscuta* (1, 11). However, the possibility of a more direct regulatory role for maternal tissues should not be overlooked (16).

The observed interaction between temperature and O_2 treatment with respect to ^{14}C uptake is consistent with the suggestion that a considerable difference may exist between the O_2 content of the external atmosphere and that of the sites of photosynthate utilization in the embryo (3, 17). An increase in fruit temperature from 25 to 35°C resulted in no net increase in ^{14}C uptake by the embryos of intact fruit exposed to 21% O_2 and only a modest 14% increase in those exposed to 100% O_2 (Fig. 2). On the contrary, the same temperature changes resulted in pronounced increases in ^{14}C uptake by isolated embryos, without the stimulation of 100% over 21% O_2 (Fig. 3) as had occurred with intact fruit (Figs. 2, 4). Within intact fruit under ambient atmospheric conditions, O_2 consumption by very rapid pod wall and seed coat respiration (2, 17) may effectively create near anaerobiosis at the embryo level (17). Also, since atmospheric O_2 must diffuse first through the pod wall and then into the seed through the small micropyle of the seed coat (26), the resistance to O_2 diffusion to the embryo may indeed be great (3). This would be expected to reduce embryo uptake of photosynthate as in anaerobic excised embryos (Fig. 3, Tables I and II), unless the O_2 gradient can be sufficiently steepened experimentally. The data suggest (Figs. 2, 4) that this is increasingly difficult above 25°C. These experiments seemingly explain the earlier observations of Quebedeaux and co-workers (20).

CONCLUSIONS

These data provide insights into the mechanisms of environmental regulation of photosynthate import in developing soybean fruit. Clearly, temperature has a profound influence on photosynthate import, perhaps both directly through effects on energy metabolism or membrane permeability, and indirectly through O_2 availability effects on fruit tissue respiration. Thus, as the temperature of unexcised (but not of excised) embryos was increased beyond 25°C, uptake of label became more O_2-dependent, perhaps in response to enhanced respiration of the surrounding maternal fruit tissues.

Exposing soybean pods to anaerobiosis prevented sucrose efflux from the seed coat at all temperatures, preventing even passive accumulation of label by the embryo. Although primary regulation of photosynthate import is thought to reside in the embryo, the extent to which phloem unloading contributes is unknown. Further studies of regulatory mechanisms of import are in progress.

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