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Sugar Efflux from Attached Seed Coats of *Glycine max* (L.) Merr.

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ABSTRACT

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Sugar efflux (sucrose + glucose) from attached seed coats of *Glycine max* (L.) Merr. was measured at high sampling rates to improve the kinetic characterization of seed coat exudation. This study confirms that sugar efflux in seed coats has at least two components, and demonstrates that the concentration of mannitol osmoticum bathing the seed coat may influence one or both of these components. High leaf irradiance increased sugar efflux relative to a low leaf irradiance at the same mannitol concentration. A high concentration of mannitol (500 mol m^{-3}) enhanced sugar efflux relative to a medium concentration (100 mol m^{-3}) under both high and low leaf irradiance. A low mannitol concentration (10 mol m^{-3}) stimulated sugar efflux (relative to 100 mol m^{-3}) to a greater extent when leaf irradiance was high. Rapid changes in mannitol concentration produced immediate stimulations of sugar efflux. Effects of osmoticum on sugar efflux are explained by simultaneous turgor-mediated effects on import of sucrose by the phloem and retrieval of apoplastic sucrose, presumably by seed coat parenchyma.

Key words—*Glycine max*, seed coat, turgor-sensitive transport, assimilate transport.

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INTRODUCTION

The symplastic separation of maternal and embryonic tissues in the legume fruit facilitates removal of the developing embryo, leaving an 'empty' seed coat which can be filled with a solution to trap unloading solutes (Thorne and Rainbird, 1983). Sucrose is quantitatively the most important compound unloaded to the apoplast of seed coats and is the focus of most studies (Thorne, 1985; Wolswinkel and Ammerlaan, 1985). The rate of sucrose exudation from attached and detached seed coats is similar for 2–4 h, which is evidence for a symplastic pool of sucrose in the seed coat that 'washes out' when the seed coat cup is filled with solution (Wolswinkel and Ammerlaan, 1985; Gifford and Thorne, 1986). After this initial 'washout' period, sucrose efflux from detached seed coats declines significantly, while efflux from attached seed coats appears to reach a steady-state (Wolswinkel and Ammerlaan, 1985; Gifford and Thorne, 1986). The steady-state sucrose efflux observed in attached seed coats after the initial 'washout' period has been attributed to phloem unloading (Wolswinkel and Ammerlaan, 1985; Gifford and Thorne, 1986).

Osmotic conditions within the seed coat cup influence the efflux of sucrose from this tissue,

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irrespective of whether sucrose or mannitol is used for osmoticum (Wolswinkel and Ammerlaan, 1984). High osmoticum levels enhance sucrose efflux, presumably through stimulation of mass flow in the phloem by low apoplast solute potentials (Wolswinkel and Ammerlaan, 1984; Wolswinkel, 1985; Minchin and McNaughton, 1986). There is evidence that the apoplastic concentration of sucrose and other osmotically active compounds is relatively high *in vivo* (Patrick, 1983; Gifford and Thorne, 1985), a finding that supports the theory that high osmoticum concentrations in the seed coat apoplast promote high levels of phloem transport in this tissue (Wolswinkel, 1985; Minchin and McNaughton, 1986).

Low osmoticum concentrations (0–50 mol m^{-3}) enhanced photosynthate unloading relative to 100 mol m^{-3} (Patrick, Jacobs, Offler, and Cram, 1986), or 250 mol m^{-3} mannitol elutions (Wolswinkel and Ammerlaan, 1986), and net efflux of sucrose and amino acids from excised seed coats and cotyledons was enhanced by 100 mol m^{-3} mannitol osmoticum relative to 250 or 400 mol m^{-3} mannitol (Wolswinkel, Kraus, and Ammerlaan, 1986). These results are explained by the inactivation of a sucrose retrieval system in the seed coat parenchyma (Wolswinkel and Ammerlaan, 1986; Wolswinkel *et al.*, 1986) or the stimulation of a turgor-sensitive exporter (Patrick *et al.*, 1986). Support for Wolswinkel's hypothesis of a sucrose retrieval system that is inhibited by low osmoticum concentrations is provided by Wyse, Zamski, and Tomos (1986), who have demonstrated that an active sucrose retrieval system in sugar beet taproot tissue is inhibited by low osmoticum concentrations. In contrast to these findings, Gifford and Thorne (1986) reported no significant difference between unloading rates at 0 and 150 mol m^{-3} osmoticum concentrations, and Minchin and McNaughton (1986) demonstrated lower rates of ^{11}C-labelled photosynthate unloading in 0 mol m^{-3} compared to 300 mol m^{-3} osmoticum elutions. Apparent conflicts between observations of stimulation versus inhibition of photosynthate efflux have been explained in terms of the relative balance between phloem transport, which is inhibited by low osmoticum concentrations, and release from the seed coat, which is stimulated by low osmoticum concentrations (Wolswinkel *et al.*, 1986). The effects of osmoticum concentration on sucrose efflux have also been observed using rapid osmoticum changes, demonstrating that osmotic stimulation or inhibition of unloading is a dynamic process (Patrick *et al.*, 1986).

Although investigations of seed coat structure have been made to determine the pathway by which unloading occurs, there is disagreement over the extent of symplastic movement within the unloading pathway (Thorne, 1981; Offler and Patrick, 1984; Thorne, 1985; Patrick *et al.*, 1986). Development of a model for photosynthate unloading in seed coats has been frustrated by controversy over the relative compartmentation of photosynthates within the seed coat (Thorne, 1985; Gifford and Thorne, 1986; Patrick *et al.*, 1986), the cellular location of photosynthate unloading to the apoplast (Thorne, 1985; Gifford and Thorne, 1986; Patrick *et al.*, 1986), and the transport mechanisms involved in this unloading (Gifford and Thorne, 1986; Minchin and McNaughton, 1986; Patrick *et al.*, 1986; Wolswinkel and Ammerlaan, 1986). In order to derive an acceptable model of phloem unloading in seed coats, more information on the compartmentation, kinetics, and osmotic relations of sucrose efflux from the seed coat must be obtained. Detached seed coats are inhibited with respect to long-term phloem unloading (Gifford and Thorne, 1986) and, therefore, are not useful for long-term studies of this process. Pulse-labelling techniques have typically been used to create a flux of labelled sucrose that is interpreted as a relative measure of cold sucrose flux through the seed coat (Patrick, 1983; Wolswinkel and Ammerlaan, 1984). Analysis of labelled photosynthate pulses travelling through legume seed coats is kinetically complex, and the relationship between labelled and cold sucrose fluxes is not necessarily stable or predictable (Thorne, 1985; Zierler, 1981). Thus, there is a need for experiments which do not employ pulse-labelling or detached seed coats. This paper presents data on the osmotic relations of

unlabelled sugar (sucrose + glucose) efflux from attached *Glycine max* seed coats using an automated perfusion system.

MATERIALS AND METHODS

Plant material

A determinate soybean (*Glycine max* (L.) Merr. cv. Portugal) with average seed dry weight of 260 mg was grown in the greenhouse in late summer, without supplemental lighting. Plants were treated with the systemic pesticide Oxamyl 2 weeks after planting, and were fertilized bi-weekly with a dilute solution of Peter's Professional Plant Food (W. R. Grace & Co., Fogelsville, PA) applied to the soil. Flowering began approximately 35 d after planting. Plants were trimmed to one three-seeded pod per node by removing extra pods as soon as the number of seeds per pod could be determined. Pods of the 5th to 10th node were studied 22 to 34 d after flowering, at which point the seeds had reached 400 to 600 mg fresh weight. Experiments were carried out either in the greenhouse under full sun (1 800 μmol m^{-2} s^{-1} at 12.00 p.m.) or in the laboratory under a 1 000 W mercury High Intensity Discharge lamp (General Electric, Cleveland, Ohio) filtered through 6·0 cm of circulating water (300 μmol m^{-2} s^{-1}). Plants were exposed to the low indoor irradiance for 2 d before use. All pods were held in an upside down position using twist ties for at least 24 h preceding the experiment to avoid thigmonastic effects from positioning the pod on the day of the experiment. Experiments were begun between 10.30 a.m. and 12.00 p.m.

Surgical procedure

To provide access to the seed coat cup, an incision was made through the dorsal suture of the pod in a procedure differing slightly from that of Thorne and Rainbird (1983). The incision removed approximately 20% of the distal side of the middle seed coat, exposing the cotyledons which were cut into pieces and removed. The empty seed coat was then rinsed for 20 s with distilled water to remove cotyledon fragments. The pod was clamped in an inverted position with a loosely fitting holder made from aluminium foil wrapped in Parafilm, and a buffered osmotic solution was perfused through the seed coat cup for 10–15 min before sampling began. To prevent drying under high light and temperature conditions (i.e. the greenhouse), the seed coat cavity was surrounded with moist tissue paper.

Perfusion technique

Perfusion solutions contained 5·0 mol m^{-3} MES-buffer, pH 6·0, and 1·0 mol m^{-3} $CaCl_2$, with various concentrations of mannitol as an osmoticum. The solution bathing the seed coat was constantly renewed by a two-channel peristaltic pump (Cole Parmer, Chicago, Illinois), coupled with an all-Teflon solenoid valve (ASCO Red Hat S062690, Automatic Switch Co., Florham Park, N.J.) (Fig. 1). One channel of the pump constantly removed perfusate for analysis, while the other channel periodically filled the seed coat cup to within a millimeter of the incision under the control of the solenoid valve (Fig. 1). The pump speed was held constant, and the frequency and duration of valve opening was modulated using an electronic timing circuit. This method could be used to fill seed coats of any volume between 100 and 500 mm^3 with an error of $\pm$ 10 mm^3. The time required to fill and empty a seed coat varied according to the seed coat cup volume, but was always close to 50 s. The seed coat was empty for approximately 3 s between filling cycles. The rate of perfusion through the seed coat was approximately 350 mm^3 min^{-1}. Perfused solution was collected as 5 min samples by a FRAC-100 fraction collector (Pharmacia, Piscataway, N.J.). During the 3 s period when the seed coat was empty, a small air bubble formed in the solution moving out of the seed coat. Thus, individual 50 s samples were observable, and could be collected if desired. It was possible to change the perfusion solution available at the input for the controlling valve, and thus the effects of osmoticum concentration changes could be studied. All tubing used in these experiments was either Teflon or Tygon.

Sugar analysis

Total sucrose + glucose in each sample was determined after sucrose hydrolysis by invertase using the glucose oxidase/peroxidase method (Wolswinkel and Ammerlaan, 1984). Sucrose is present at much higher concentrations than glucose in seed coat exudates of *Glycine max* (Gifford and Thorne, 1986). Elution rates were calculated as nmol sucrose + glucose per seed coat per minute. Cumulative data were obtained by adding the amount of sugar (sucrose + glucose) in each sample to the sum of all sugar eluted in the preceding samples.

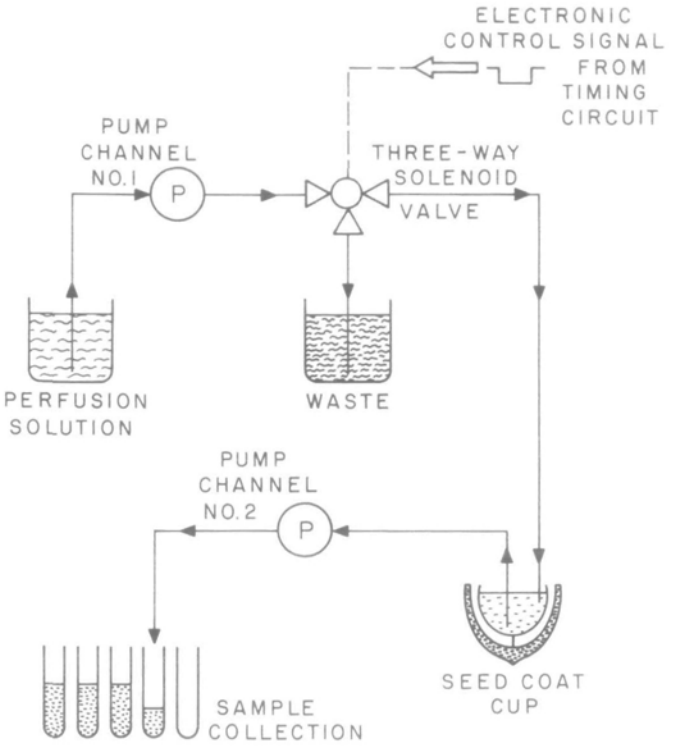

FIG. 1. Schematic diagram of seed coat perfusion apparatus. A three-way solenoid valve directs perfusion solution into the seed coat cup when signal from timing circuit is high, otherwise solution is diverted to waste. Pump channel number 1 has a high flow rate (large diameter tubing), and pump channel number 2 has a low flow rate (small diameter tubing).

RESULTS

General characteristics of sugar efflux over time

During the first 2 h of elution from attached seed coats, a large sugar efflux (sucrose + glucose) was observed (Fig. 2). This large initial efflux decreased rapidly to a lower, more stable rate which continued for many hours, with a tendency for efflux to approach zero after about 12 h (Fig. 3). Because variability between replicates was relatively small compared to the difference between treatments, data from two experiments were combined. The initial 10 min period of very high efflux (phase one) described by Gifford and Thorne (1986) was not observed in these experiments because no samples were collected until 15 min after perfusion had begun. The remaining two efflux periods described by Gifford and Thorne (1986) were observed: a 2 to 3 h period of moderately high sugar efflux which declined rapidly (phase two), and a 3 to 4 h period of relatively low efflux which declined slowly (phase three) (Figs 2, 3). The second phase of efflux has been described as a washout of sucrose from the seed coat symplasm which takes place during the first 2 h of elution (Gifford and Thorne, 1986), and the third phase has been attributed to sucrose efflux from the phloem (Wolswinkel and Ammerlaan, 1985; Thorne, 1985; Gifford and Thorne, 1986).

Osmotic relations of long-term sugar efflux

Comparison of sugar exudation rates from attached seed coats under different osmotic conditions demonstrated that the osmotic environment influences sugar efflux (Fig. 2). Leaf

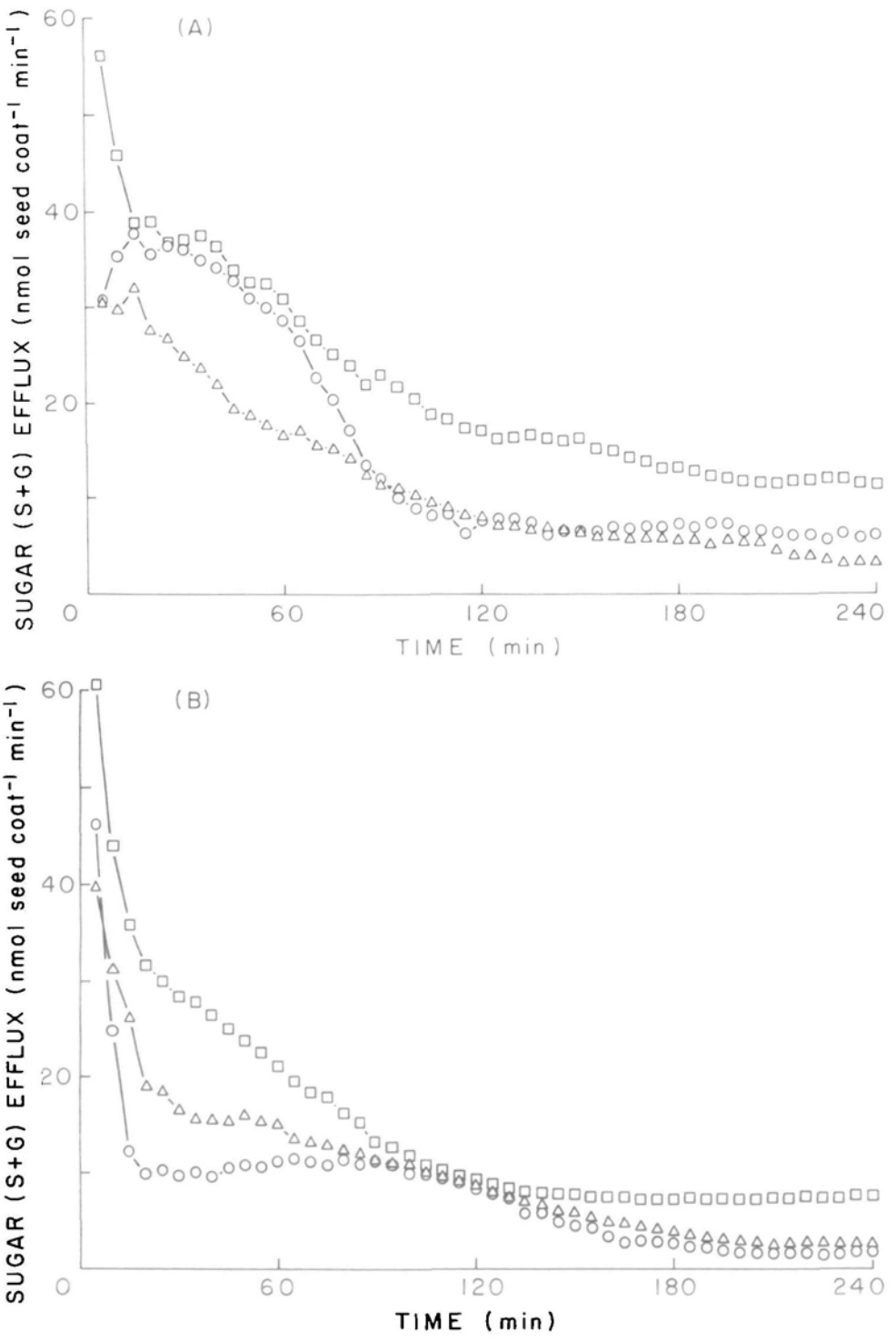

FIG. 2. Effect of three mannitol concentrations (10 mol m^{-3}, ○; 100 mol m^{-3}, △; 500 mol m^{-3}, □) on the rate of sugar (sucrose + glucose) release from attached seed coats during a 4 h elution period. Each curve represents data from two experiments averaged. Two leaf irradiance levels were used: 1 800 μmol m^{-2} s^{-1} (A), and 300 μmol m^{-2} s^{-1} (B).

irradiance also influenced sugar efflux, with average effluxes under high irradiance being considerably greater than effluxes under low irradiance (Table 1; Figs 2, 4). High leaf irradiance also produced consistently higher effluxes during phase three than did low leaf irradiance at comparable mannitol concentrations (Table 1; Fig. 2). Because of the large influence that light conditions exert on seed coat response to osmotic environment, it is necessary to describe results in relation to both high and low light observations.

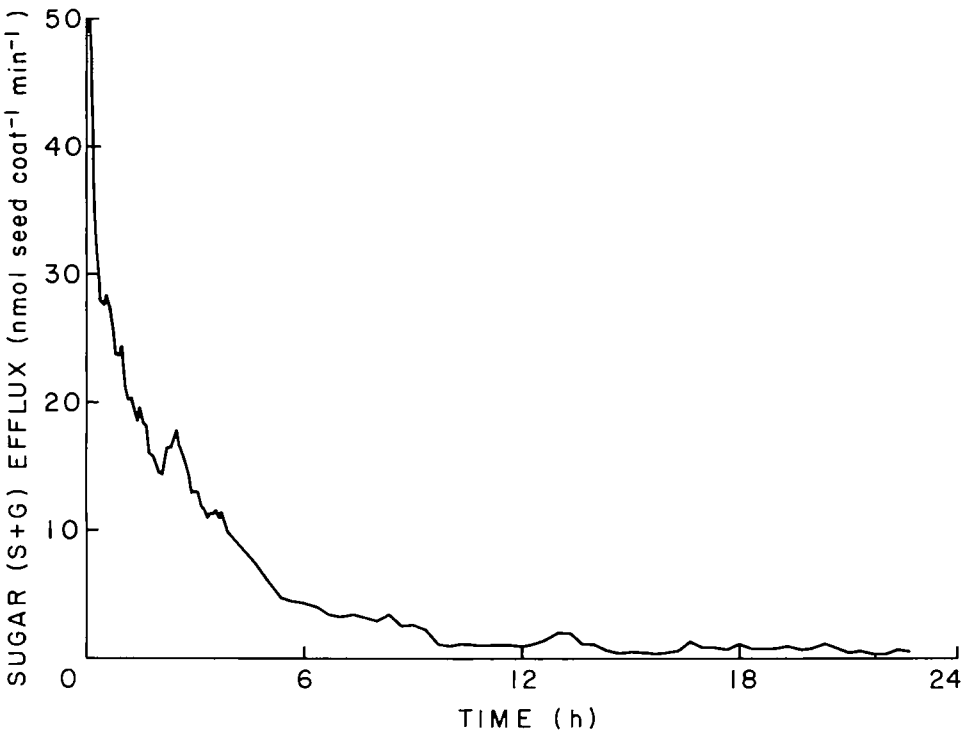

FIG. 3. Rate of sugar (sucrose + glucose) release from an individual attached seed coat during a typical 24 h elution period. Samples were taken at 5 min intervals. The mannitol concentration in the perfused solution was 500 mol m^{-3}, and leaf irradiance was 1 800 μmol m^{-2} s^{-1}.

Graphs of cumulative sugar release demonstrated that high mannitol concentrations (500 mol m^{-3}) increased the total sugar efflux and the third phase of efflux above that of lower mannitol concentrations regardless of light conditions (Table 1; Figs 2, 4). Under low leaf irradiance, sugar effluxes into 100 mol m^{-3} mannitol were larger than into 10 mol m^{-3} mannitol. At a high leaf irradiance, 10 mol m^{-3} mannitol osmoticum produced a larger total sugar efflux than did 100 mol m^{-3} mannitol (Table 1; Fig. 4). The third phase sugar effluxes of 10 mol m^{-3} mannitol elutions relative to 100 mol m^{-3} mannitol elutions parallel the total effluxes observed, under both high and low leaf irradiance (Table 1; Fig. 2). In summary, lower mannitol concentrations produced lower sugar effluxes than did higher mannitol concentrations under low leaf irradiance, but, under high irradiance, 10 mol m^{-3} mannitol osmoticum produced a higher sugar efflux than 100 mol m^{-3}. Intermediate osmoticum concentrations (100 or 200 mol m^{-3}) produced intermediate effluxes under low irradiance, and lower effluxes under high irradiance.

TABLE 1. *Effects of osmotic environment and leaf irradiance on sugar (sucrose + glucose) efflux from attached seed coats of* Glycine max

Data are the average of two experiments.

Mannitol concentration in perfused solution (mol m^{-3})	Sugar (S + G) efflux					
	Leaf irradiance 1 800 μmol m^{-2} s^{-1}			Leaf irradiance 300 μmol m^{-2} s^{-1}		
	Eluted[a] sugar (μmol)	Phase 3[b] efflux (nmol min^{-1})	Washout[c] sugar (μmol)	Eluted[a] sugar (μmol)	Phase 3[b] efflux (nmol min^{-1})	Washout[c] sugar (μmol)
10	3·62	6·0	2·19	1·87	1·6	1·48
100	2·79	3·3	1·99	2·39	2·7	1·75
200[d]	—	—	—	2·62	4·0	1·68
500	5·23	11	2·41	3·61	7·5	1·81

[a] Total sugar eluted from a single seed coat in a 4 h period (μmol).
[b] The rate of sugar exudation from a single seed coat at the end of a 4 h elution period (nmol min^{-1}). Represents the mean of five points.
[c] Calculated by subtracting the total amount of sugar exuded at the phase three rate (assuming a constant rate for 4 h) from the total amount of sugar eluted during a 4 h period (μmol).
[d] These values represent only one experiment.

Phloem unloading and the components of sucrose efflux

To analyse the kinetics of sugar efflux versus time, an attempt was made to separate sugar efflux into two components. Glucose release does not contribute significantly to total sugar release and is not kinetically significant in relation to sucrose efflux (Gifford and Thorne, 1986). For this reason, sucrose + glucose measurements may be described as sucrose fluxes. It may be assumed that the phase three sugar efflux is fundamentally distinct from other sugar effluxes in the seed coat if it represents a phloem unloading component (Gifford and Thorne, 1986). Subtraction of the phase three sugar efflux from all values of sugar efflux in an experiment should produce data representing the phase two efflux (symplast washout) of sucrose. Thus, sugar 'washout' efflux was obtained by subtracting the mean of the last five points in each 4 h experiment from each value of sugar elution rate in the experiment. Although this procedure is based on the assumption that the phase three efflux is constant throughout an experiment, the total 'washout' sugar efflux calculated appears to be relatively insensitive to mannitol concentration as expected in an ideal tissue washout (Table 1).

Kinetic analysis

The time course of solute efflux from compartments within a tissue undergoing an ideal washout can be described by first order exponential functions, assuming that resistances to solute movement within a tissue are independent of time and solute concentration. Non-ideal conditions, such as heterogenous distribution of material within a compartment, non-instantaneous mixing of material within a compartment, physiological stimulation of efflux or influx, and physical limitations to solute movement, complicate first order efflux kinetics (Zierler, 1981). First order kinetics are visible in logarithmic plots of solute efflux versus time as a series of distinct linear components with more negative slopes preceding less negative ones. Logarithmic plots of seed coat sugar efflux versus time (Fig. 5) demonstrate some adherence to first order kinetics, but also show a great deal of variation in response to differing osmoticum concentrations. The most obvious deviations from first order kinetics in

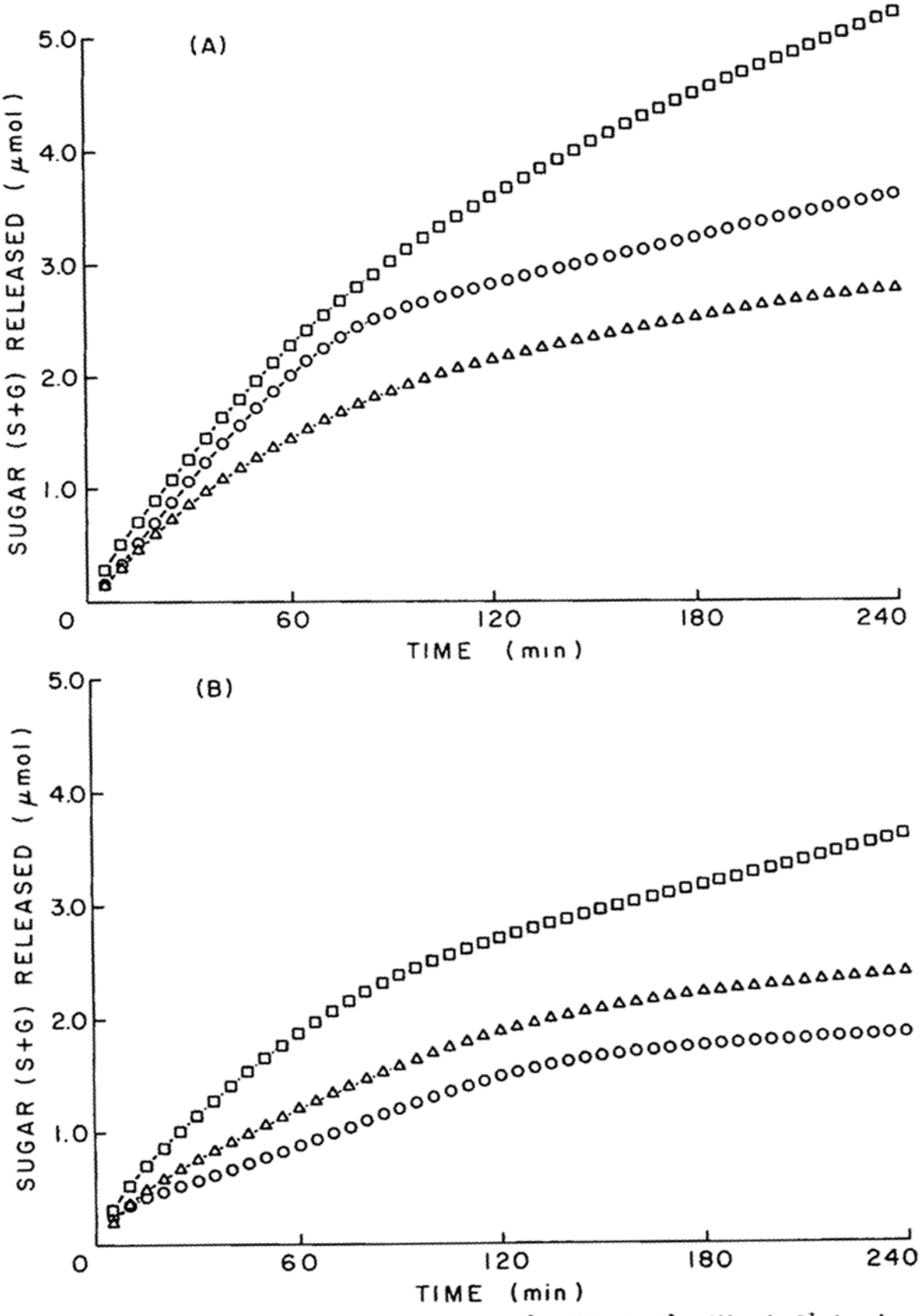

FIG. 4. Effect of three mannitol concentrations (10 mol m^{-3}, ○; 100 mol m^{-3}, △; 500 mol m^{-3}, □) on the cumulative sugar (sucrose + glucose) release from attached seed coats during a 4 h elution period. Each curve represents data from two experiments averaged. Two leaf irradiance levels were used: 1 800 μmol m^{-2} s^{-1} (A), and 300 μmol m^{-2} s^{-1} (B).

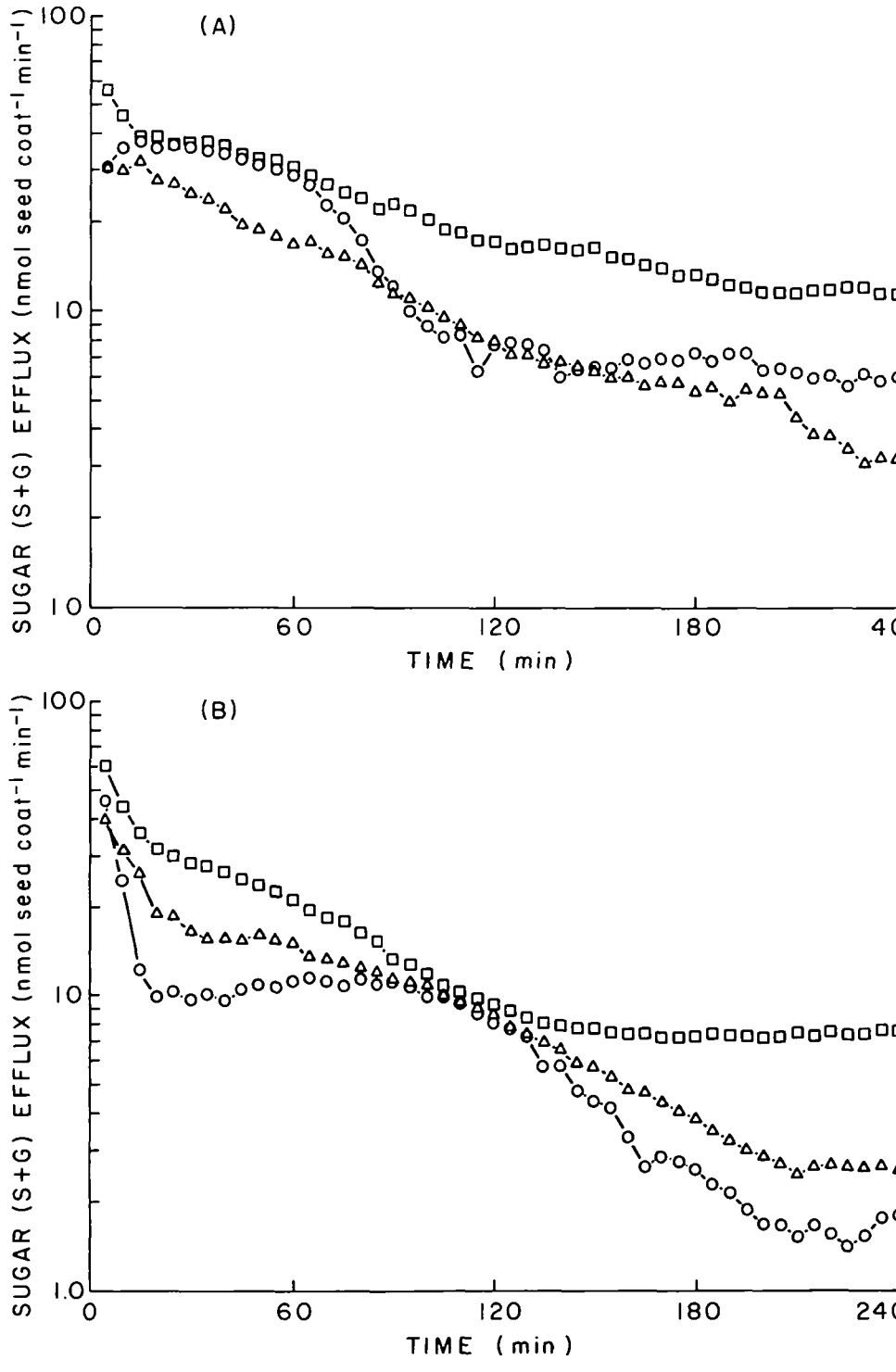

FIG. 5. Effect of three mannitol concentrations (10 mol m^{-3}, o; 100 mol m^{-3}, Δ; 500 mol m^{-3}, □) on the rate of sugar (sucrose + glucose) release from attached seed coats during a 4 h elution period (logarithmic plot of Fig. 1). Each curve represents data from two experiments averaged. Two leaf irradiance levels were used: 1 800 μmol m^{-2} s^{-1} (A), and 300 μmol m^{-2} s^{-1} (B).

logarithmic plots of sugar efflux versus time were observed using 10 mol m^{-3} mannitol under both high and low light. This effect was present in the form of a 'hump-shaped' graph. Elutions into 500 and 100 mol m^{-3} mannitol gave logarithmic plots that do not deviate greatly from linearity (Fig. 5), and also give evidence of more than one linear component of efflux, which would be expected of a multi-compartment system. In summary, logarithmic plots of sugar efflux using 10 mol m^{-3} mannitol show more deviation from first order kinetics than do logarithmic plots of elutions with higher concentrations of mannitol.

Changes in sugar efflux induced by changes in osmoticum concentration

After the seed coat sugar efflux had reached phase three (approximately 4 h), it was possible to stimulate or inhibit the efflux by changing the mannitol concentration in the perfusion

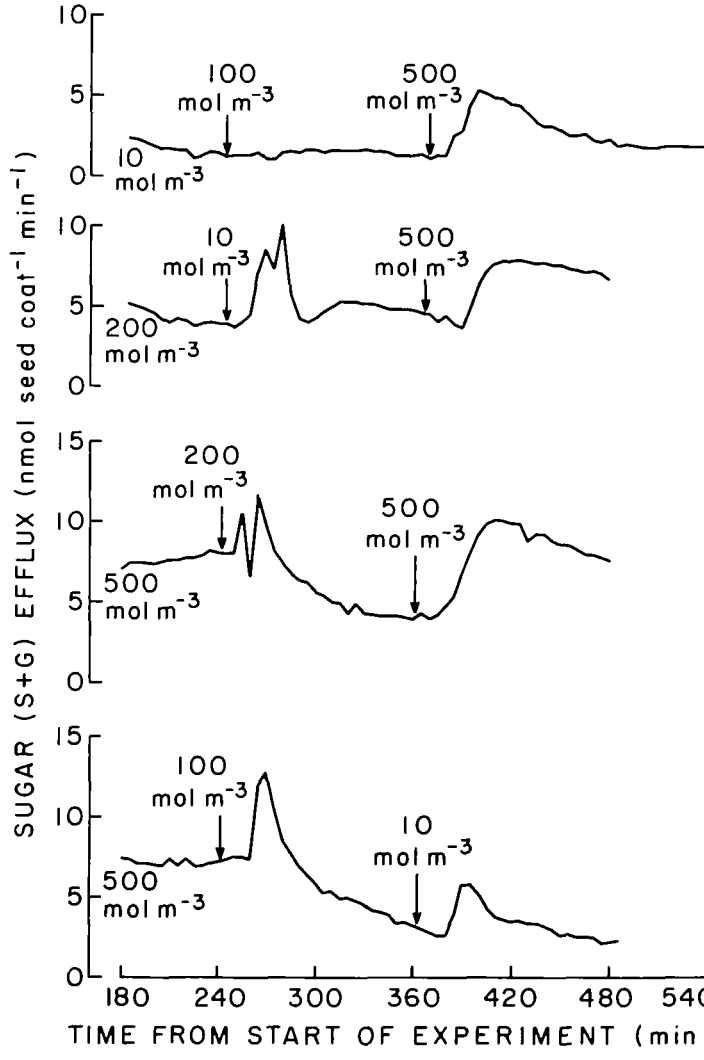

FIG. 6. Effect of mannitol concentration changes on the rate of sugar elution from attached seed coats. Each curve represents one experiment and the time-scale is relative to the start of elution. Samples were taken at 5 min intervals. The initial mannitol concentration is indicated at the beginning of each experiment with concentration changes indicated by the arrows. Leaf irradiance was 1 800 μmol m^{-2} s^{-1}.

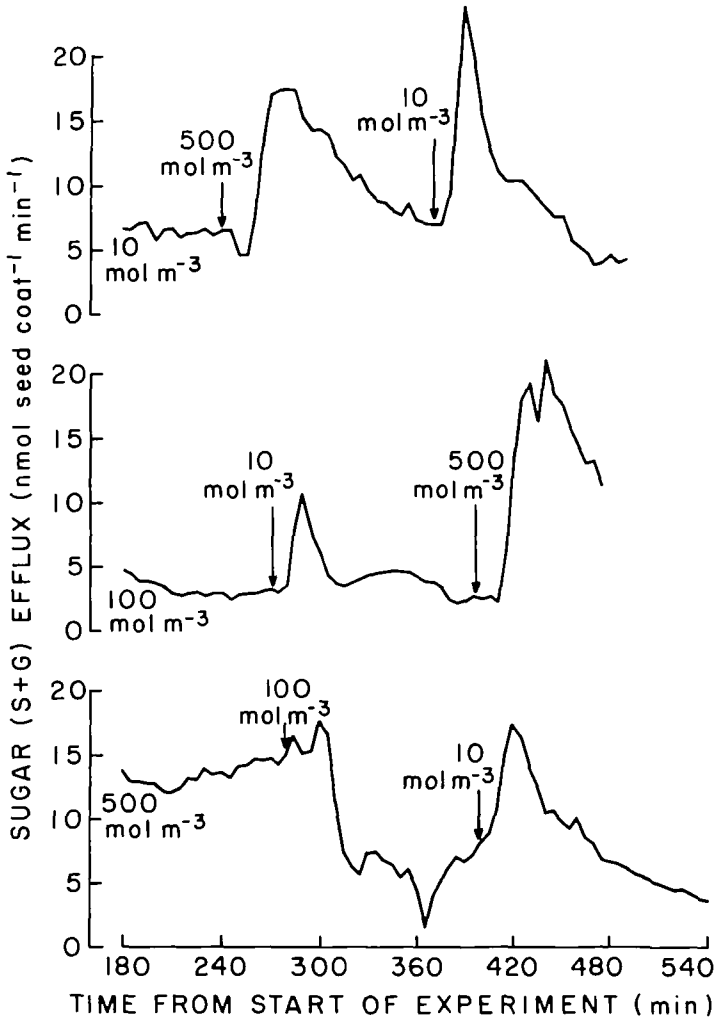

FIG. 7. Effect of mannitol concentration changes on the rate of sugar elution from attached seed coats. Each curve represents one experiment, and the time scale is relative to the start of elution. Samples were taken at 5 min intervals. The initial mannitol concentration is indicated at the beginning of each experiment with concentration changes indicated by the arrows. Leaf irradiance was 300 μmol m^{-2} s^{-1}.

solution (Figs 6, 7). The most common response to changing the concentration of mannitol bathing the seed coat was rapid stimulation of sugar efflux that can best be described as a 'peak' in the efflux (Figs 6, 7). Changing from a low or intermediate mannitol concentration to a high mannitol concentration (500 mol m^{-3}) induced a large sustained peak in the efflux irrespective of light conditions, a result which is similar to long-term stimulation of efflux by high mannitol concentrations (Figs 6, 7). Similarly, changing from a high (500 mol m^{-3}) or intermediate (100 or 200 mol m^{-3}) mannitol concentration to a low (10 mol m^{-3}) mannitol concentration typically produced a smaller, short duration peak in the sugar efflux (Figs 6, 7) followed by a lower steady rate. Under high irradiance, changing from a high mannitol concentration (500 mol m^{-3}; Fig. 6) or from a low mannitol concentration (10 mol m^{-3}; data not shown) to an intermediate concentration (100 mol m^{-3}) inhibited sugar efflux. Under low irradiance (Fig. 7), changing from a high mannitol concentration (500 mol m^{-3}) to an

intermediate concentration (100 or 200 mol m^{-3}) produced a small peak, followed by a large reduction in efflux. Changing from a low mannitol concentration (10 mol m^{-3}) to an intermediate mannitol concentration (100 or 200 mol m^{-3}) had no significant effect on sugar efflux (Fig. 7). These results from osmoticum change experiments parallel long-term effluxes observed using the same mannitol concentrations. This can be seen by comparing the phase three effluxes observed during long-term elutions (Table 1) with the relative effluxes produced by changes in mannitol concentration (Figs 6, 7).

The effluxes produced by rapid osmoticum concentration changes were not always comparable to the effluxes observed in long-term elutions. The most obvious deviation from this comparison is the stimulatory effect produced by changing from 500 mol m^{-3} mannitol osmoticum to 10 mol m^{-3} mannitol, at a high leaf irradiance (Fig. 6). Similarly, 10 mol m^{-3} mannitol induced a peak in the sugar efflux when preceded by 200 or 100 mol m^{-3} mannitol under low leaf irradiance (Fig. 7). It was also apparent that the initial and long-term effects of an osmoticum change could differ. For example, an initial stimulation may give rise to a long-term decrease in sugar efflux (Figs 6, 7).

DISCUSSION

Leaf irradiance and sugar efflux

It was found that leaf irradiance has long-term effects on the sugar flux in seed coats. The influence of leaf irradiance on sugar efflux kinetics may be explained by changes in seed coat sucrose concentrations caused by changes in source strength. For example, sugar efflux (both total and phase three) stimulation by high leaf irradiance (Table 1; Figs 2, 4) may be explained by an increase in source strength.

Osmotic effects on the sugar efflux

The observed stimulation of long-term (phase 3) sucrose efflux by high mannitol concentrations, regardless of leaf irradiance, may be attributed to the acceleration of phloem transport by a low solute potential in the apoplast (Wolswinkel, 1985; Minchin and McNaughton, 1986).

Under high irradiance, seed coats perfused with low osmoticum concentrations exhibited higher sugar effluxes than those perfused with intermediate osmoticum concentrations (Table 1; Fig. 2). However, this stimulation was eliminated by 2 d of low leaf irradiance (Table 1).

Stimulation of net sugar efflux by low osmoticum concentrations has been explained by the inhibition of sucrose retrieval from the seed coat apoplast (Wolswinkel and Ammerlaan, 1986). Although the kinetics of the retrieval system are not known, it can be assumed, by analogy with the beet system (Wyse *et al.*, 1986), that the turgor-sensitive component of retrieval is saturated at apoplast sucrose concentrations above 15 mol m^{-3}. Continuous perfusion should lower the sucrose concentration in the seed coat apoplast below the level present in the intact seed coat (170 mol m^{-3}; Gifford and Thorne, 1985). Low leaf irradiance decreased the sugar efflux from the seed coat (Table 1), and this would reflect a correspondingly lower apoplast sucrose concentration. It is likely that the apoplast sucrose concentration in continuously perfused seed coats from plants under low leaf irradiance was significantly lower than the saturation level for turgor-sensitive sucrose retrieval. At apoplastic sucrose concentrations below the saturation level, sucrose retrieval would be significantly reduced, and turgor-inhibition of retrieval would be correspondingly less. This may explain why sugar efflux stimulation by low osmoticum concentrations was not observed at low leaf irradiance (Table 1).

Deviation from first-order kinetics

The large deviation from first-order kinetics observed in logarithmic plots of sugar efflux at low osmoticum concentrations may indicate a fundamental change in the basic parameters of efflux, such as inhibition of sucrose retrieval (Fig. 5). The 'hump-shaped' graph of sugar efflux observed at low mannitol concentrations (Figs 2, 5) demonstrates that efflux stimulation is most prominent after net sugar efflux had declined to some extent.

Rapid changes in sugar efflux

Observation of rapid changes in sugar efflux in response to changes in osmoticum concentration (Figs 6, 7) demonstrated that turgor-sensitive sugar efflux is a dynamic process (Patrick *et al.*, 1986). Changing from low or intermediate (10, 100, and 200 mol m^{-3}) to high mannitol concentrations (500 mol m^{-3}) produced large transient stimulations of sugar efflux (Figs 6, 7). However, sugar efflux could also be transiently stimulated by changing from high or intermediate mannitol concentrations (500, 200, and 100 mol m^{-3}) to low mannitol concentrations (10 mol m^{-3}). This observation provides evidence for more than one mechanism of osmoticum-induced sugar efflux stimulation: one sensitive to high mannitol concentrations, and one sensitive to low mannitol concentrations. The existence of more than one mechanism of sugar efflux stimulation is also supported by the observation of differences between the immediate and long-term effects of an osmoticum change. The variety of observed responses to osmoticum changes bespeaks the complexity of describing these effects. The most significant result of the osmoticum change experiments is the demonstration that sugar efflux is controlled by mechanisms capable of producing large and immediate efflux changes in response to the osmotic environment of the seed coat apoplast.

CONCLUSIONS

The ability to observe sucrose efflux from attached seed coats at high sampling frequencies provides greater detail for kinetic analysis. The findings of this study add to the work of Gifford and Thorne (1986) in confirming that sucrose efflux in seed coats has at least two components, and further demonstrate that the osmotic environment of the seed coat may influence these components. The osmotic effects on sugar efflux observed in these experiments can be explained by the combination of phloem transport stimulation by high osmoticum concentrations and apoplast sucrose retrieval inhibition by low osmoticum concentrations. Integration of phloem sucrose transport and apoplast sucrose retrieval in the seed coat is capable of producing sugar efflux stimulation at both high and low osmoticum concentrations as described by Wolswinkel *et al.* (1986). In addition, it has been shown that leaf irradiance influences the effects of osmoticum on sugar efflux, and that rapid changes in mannitol concentration are capable of producing both stimulations and inhibitions of sugar efflux from attached seed coats. The inherent complexity of sucrose fluxes in legume seed coats limits the scope of unlabelled sugar elution experiments in the study of phloem unloading, unless coupled with an independent method of distinguishing between phloem unloading and the efflux of sucrose from compartments within the seed coat. Long-term sugar efflux experiments do not provide adequate evidence to determine the physiological and kinetic significance of the different components of seed coat sugar efflux that have been observed. However, coupling this technique with carefully controlled tracer experiments may hold promise for future research.

ACKNOWLEDGEMENTS

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