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Further Comparisons between Carbowax 6000 and Mannitol as Suppressants of Cucumber Hypocotyl Elongation

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ABSTRACT

Stirring largely eliminated the greater effectiveness of Carbowax 6000 compared to mannitol in reducing cucumber (*Cucumis sativus* L.) hypocotyl section elongation. Stirring increased elongation in water but reduced elongation in growth regulator solution. Shrinkage of cells without plasmolysis in hypertonic Carbowax solutions indicates that Carbowax 6000 did not penetrate cell walls. Sections prevented from elongating during pretreatment exposure to saturated air grew as much as those not pretreated. Sections pretreated with isotonic Carbowax responded similarly, but sections pretreated with isotonic mannitol grew less.

An earlier report (10) indicated that elongation of cucumber hypocotyl sections in growth regulator solution was suppressed more by Carbowax 6000 than by mannitol at equivalent water potentials. Carbowax 6000 and mannitol reduced respiration comparably. Pretreatment with mannitol reduced subsequent growth more than pretreatment with Carbowax 6000. Packer (12) extended comparisons between mannitol and Carbowax 6000. She suggested, but did not show, that boundary layers might cause the differences in elongation noted for mannitol and Carbowax solutions at equivalent water potentials. She also reported extensive data confirming more rapid deterioration of sections in mannitol than in isotonic Carbowax.

In the work reported here, growth media containing osmotica were stirred to determine whether reduction of boundary layers would largely eliminate differences in growth responses to the two osmotica. The observation of different shrinkage in hypertonic Carbowax from that in hypertonic mannitol stimulated additional experimentation directed toward explaining this difference. During exposure to any osmoticum, deterioration of the ability of sections to elongate might reflect side effects rather than water potential effects of the osmoticum; therefore, a means of suppressing growth without introducing osmotica, inhibitors, anaerobic conditions, or lowered temperature was sought. Suppressing growth by holding sections in saturated air was chosen as a standard for comparison in estimating possible deleterious effects of exposure to Carbowax and mannitol.

MATERIALS AND METHODS

Asgrow Vigorpak cucumber (*Cucumis sativus* L. Marketer) seedlings were grown in sand in a 25 C darkroom. Phototropically inactive green light was used as necessary. On the 5th day a 10-mm section was cut from each hypocotyl, beginning 2 mm below the top of the hook.

Sections to be stirred were cut into an empty dish before being transferred into a 50-ml Erlenmeyer flask containing 20 ml of water or solution. The flask was stoppered and clamped in a horizontal position to a vertical wheel, which was rotated at 12 rpm.

Sections to be held in saturated air were cut onto plastic screening placed over wet filter paper in the bottom of a small Petri dish and covered with a lid lined with wet filter paper. The small dish was placed inside a larger one.

Growth regulator solutions, with GA_7 substituted for GA_3, contained μM IAA, 0.1 mM GA_7, 0.1 mM $CoCl_2$, and 10 mM KCl (10). Length differences were tested for significance as indicated previously (10).

RESULTS

In growth regulator solution, stirring largely eliminated the differences between Carbowax and mannitol (Fig. 1); however, the small differences at −4, −5, −6, −8, −9, and −10 bars were significant at the 1% level. In the absence of growth regulators, stirring eliminated differences except at potentials below −6 bars (Fig. 2). Hypertonic solutions of mannitol caused greater length reductions than comparable solutions of Carbowax 6000.

Stirring, itself, affected elongation. Comparison of previous results (10) with present results (Fig. 1) indicated that stirred sections in growth regulator solution elongated appreciably less than unstirred sections. This indication was verified (Table I). The reverse occurred in the absence of growth regulators. On water, unstirred sections elongated 3.17 ± 0.25 (2 SE) mm in 30 hr; stirred sections elongated 4.71 ± 0.53 mm. Another complication from stirring was that sections in growth regulators and −6 bars mannitol nearly maintained original length when unstirred (10) but elongated an average of 0.43 mm when stirred (Fig. 1). No attempt was made to explain the effects of stirring.

The percentage reduction in elongation was greater in unstirred than in stirred solutions of Carbowax, but not in corresponding mannitol solutions (Table I). Water uptake by growing sections tended to concentrate Carbowax and mannitol in boundary layers adjacent to the sections; however, the small mannitol molecules diffused away much more rapidly than the large Carbowax molecules.

To investigate the greater shrinkage in length in hypertonic mannitol than in Carbowax, sections were placed in −10 and −18 bar solutions of mannitol and Carbowax. Sections were weighed and their volumes were measured by water displacement before and after 16-hr exposure to osmotica and following a 6-hr recovery period in water. Because measurement of

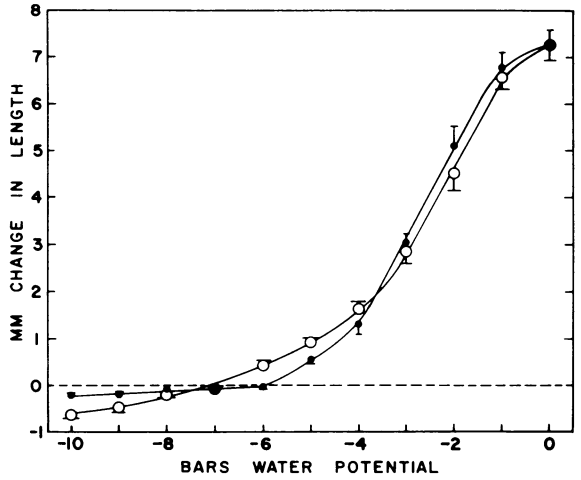

FIG. 1. Effects of decreasing water potentials by additions of Carbowax 6000 or mannitol on cucumber hypocotyl section elongation in a solution of growth regulators (IAA + GA_7 + $CoCl_2$ + KCl). Flasks containing sections were rotated during a 30-hr treatment period. Bars at data points indicate 2 SE. $N = 15$. ●: Carbowax 6000; ○: mannitol.

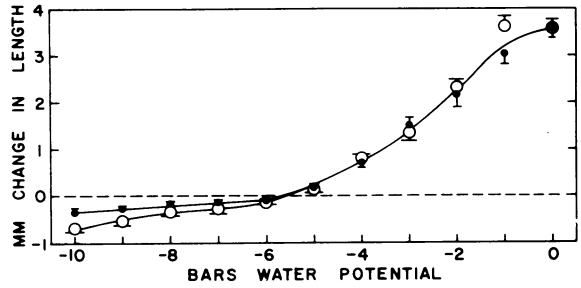

FIG. 2. Effects of decreasing water potentials by additions of Carbowax 6000 or mannitol on cucumber hypocotyl section elongation in water. Flasks containing sections were rotated during a 30-hr treatment period. Bars at data points indicate 2 SE. $N = 15$. ●: Carbowax 6000; ○: mannitol.

volume was not as accurate as that of weight and because the average densities measured within 3% of 1.05, weight was used as an indicator of volume. The results are presented in Table II. The shrinkage in length found in both mannitol and Carbowax of −10 bars was less than reported in Figures 1 and 2. Probably the exposure to water during initial volume measurement permitted a rapid increase in turgor pressure that resulted in plastic deformation. Stretched sections would not be expected to reach as short a final length in hypertonic solutions as unstretched sections.

Microscopic examination of cucumber hypocotyl sections revealed moderate plasmolysis in −10 bars and severe plasmolysis in −18 bars mannitol, and the different degrees of plasmolysis were accompanied by different size reductions (Table II). No plasmolysis was observed in Carbowax solutions of −10 and −18 bars. Even so, the water potentials of the Carbowax did affect the amounts of shrinkage (Table II). Length reduction was less but diameter reduction was much greater in Carbowax than in mannitol. Guard cells were plasmolyzed and stomata closed in mannitol. Guard cells were not plasmolyzed in Carbowax and stomata were open, some even being circular in appearance.

Elodea (*Egeria densa* Planch.) leaves immersed in mannitol solutions of −11 and −18 bars showed moderate and severe plasmolysis while only a few cells plasmolyzed in Carbowax solutions of the same potentials.

Root hairs of a 2-day-old ryegrass (*Lolium* sp.) seedling germinated on the surface of water were observed. Cyclosis occurred in water. With the introduction of −18 bars Carbowax solution, rapid flattening and twisting of the root hairs occurred, reminiscent of responses described by Jackson (8). Cyclosis apparently stopped, but no plasmolysis could be seen. The reintroduction of water brought rapid return of root hairs to their original form, and cyclosis was again noted. As −18 bars mannitol solution was introduced, there was collapse followed rapidly by return of the cell walls to their original shape but with severe plasmolysis of the protoplasts into lumps connected by strands. The final appearance was just as described by Jackson (8) for 0.5 M sucrose; however, he did not describe the momentary collapse of the root hair cell wall. Replacement of mannitol by water did not quite deplasmolyze most root hairs; the cytoplasm appeared more granular than before exposure to mannitol, and no cyclosis was observed. Obviously water diffused through the cell walls faster than mannitol, but mannitol did cross the walls rapidly, and Carbowax 6000 did not cross at all.

Cucumber hypocotyl sections held in saturated air for as long as 8 hr did not diminish in their ability to elongate in growth regulator solution (Table III). Because the response of sections exposed to isotonic Carbowax solution closely approached that of those held in saturated air, the greater elongation after exposure to isotonic Carbowax than to isotonic mannitol could hardly have resulted from stimulatory effects of Carbowax. Therefore, the reduced elongation following exposure to mannitol must have resulted from deleterious effects of mannitol.

DISCUSSION

The greater elongation in mannitol compared to Carbowax in the −4 to −6 bars range in the presence of growth regulators (Fig. 1) probably reflected the ability of sections to recover better in isotonic or near isotonic mannitol than in Carbowax. Cucumber hypocotyl sections in unstirred isotonic osmotica also grew more in mannitol than in Carbowax (12). Oat coleoptile sections in IAA and mannitol of sufficient con-

Table I. *Elongation of Cucumber Hypocotyl Sections in Unstirred and Stirred Solutions of Mannitol and Carbowax 6000*

Sections were treated for 30 hr with solutions containing growth regulators and mannitol or Carbowax 6000 and then measured. Different letters indicate significance at the 1% level in Duncan's test. $N = 15$, except for stirred solutions of −2 and −4 bars, where $N = 30$.

Osmoticum Water Potential	Stirred	Mannitol		Carbowax	
		Elongation	Reduction by osmoticum	Elongation	Reduction by osmoticum
bars		*mm* ± 2 SE	%	*mm* ± 2 SE	%
0	−	9.82 ± 0.32 a	0	9.82 ± 0.32 a	0
	+	7.66 ± 0.44 b	0	7.66 ± 0.44 b	0
−2	−	5.95 ± 0.39 c	39	5.13 ± 0.56 d	48
	+	4.63 ± 0.20 d	40	5.04 ± 0.23 d	34
−4	−	2.15 ± 0.13 e	78	1.16 ± 0.11 g	88
	+	1.86 ± 0.06 e	76	1.42 ± 0.08 f	81

Table II. *Shrinkage of Cucumber Hypocotyl Sections in Hypertonic Solutions of Mannitol and Carbowax 6000 and Recovery in Water*

Sections were cut to a length of 10.0 mm and weighed (row I); following a 16-hr treatment with osmoticum, sections were measured and reweighed (row II); after an additional 6-hr recovery period on water, sections were remeasured and reweighed (row III). Average diameter was calculated assuming volume = weight. Different letters indicate significance at the 1% level in Duncan's test. $N = 9$ or 10.

Treatment		Mannitol			Carbowax		
		Length	Weight	Diameter	Length	Weight	Diameter
		mm ± 2 SE	*mg*	*mm*	*mm* ± 2 SE	*mg*	*mm*
−10 Bars	I	10.0 c	17.7	1.50	10.0 c	17.4	1.49
	II	9.62 ± 0.07 d	15.8	1.45	9.86 ± 0.04 c	12.6	1.28
	III	10.07 ± 0.06 c	16.9	1.46	11.14 ± 0.14 a	19.5	1.49
−18 Bars	I	10.0 c	17.2	1.48	10.0 c	18.4	1.53
	II	9.32 ± 0.09 e	14.7	1.42	9.57 ± 0.06 d	7.9	1.03
	III	9.90 ± 0.00 c	15.2	1.40	10.72 ± 0.26 b	17.5	1.44

centration to stop elongation or cause initial shrinkage recovered and grew (1, 11, 13).

Cleland (2) reported greater response of oat coleoptile sections to IAA in mannitol solution if the IAA and mannitol were added simultaneously than if the mannitol was added before the IAA and suggested that responses to IAA might precede establishment of osmotic equilibrium. Although responses before attainment of osmotic equilibrium may have occurred, equilibrium should have been reached at least as soon in mannitol as in Carbowax; therefore, the greater growth in mannitol than in Carbowax could not have resulted from responses to growth regulators before osmotic equilibrium was reached.

The greater growth in mannitol compared to Carbowax at −4 to −6 bars depended on the presence of growth regulators because, in their absence, this difference did not occur (Fig. 2). Osmotic adjustment, possibly depending on the uptake of K^+ from the growth regulator solution, must have occurred in mannitol but not in Carbowax. The occurrence of osmotic adjustment is supported by the shift in isotonic concentration from −5.5 bars mannitol in water to −7.0 bars mannitol in growth regulator solution. A similar shift did not occur in Carbowax 6000. Additional support is found in the osmotic adjustment reported (9) in the xylem of roots exposed to Carbowax 400 but not to Carbowax 6000.

The different shrinkage patterns in hypertonic mannitol and Carbowax and the plasmolysis in mannitol but not in Carbowax must result from different cell wall permeability to these osmotica. Mannitol moves readily into and through cell walls, though not as rapidly as water (13) (and root hair phenomena described in the present report). Carbowax 6000 must be too large to penetrate cell walls. If it did, plasmolysis would occur. Tarkow *et al.* (14) reported that Carbowaxes of 3000 molecular weight and higher failed to enter cell walls from the lumina of green spruce heartwood. Collander (3) showed greatly decreasing permeability of the cell wall of *Nitella* as the molecular sizes of Carbowax increased from 400 through 600 to 1000. Jackson (8) described slight protoplast separation from the wall and flattening of root hairs in Carbowax 1000 that can be interpreted as resulting from little and slow penetration of the walls by the Carbowax. Flowers and Dessimoni Pinto (5) found cell walls of potato tubers much less permeable to Carbowax 1540 than to mannitol. The results of Kaufmann and Eckard (9) are consistent with cell wall permeability to Carbowax 400 but not to Carbowax 6000.

Hypertonic solutions in which the solute does not penetrate the cell wall would create negative wall pressures not found during plasmolysis. The long cells of the cucumber hypocotyl sections must be distorted more laterally than longitudinally, accounting for the much greater percentage loss in diameter than in length.

Table III. *Effect of Pretreatments with Saturated Air, Isotonic Mannitol, and Isotonic Carbowax 6000 on Elongation of Cucumber Hypocotyl Sections in Growth Regulator Solution*

Sections were pretreated 4 or 8 hr with saturated air, isotonic mannitol, or isotonic Carbowax 6000; measured; transferred to growth regulator solution for 30 hr; and remeasured. Different letters indicate significance at the 1% level in Duncan's test. $N = 15$.

Pretreatment	Length of Pretreatment	Total Elongation	
		During pretreatment	After 30 hr on growth regulators
	hr	*mm* ± 2 SE	
Saturated air	4	0.09 ± 0.03 d	10.20 ± 0.36 a
	8	0.13 ± 0.04 d	10.07 ± 0.45 a
−5.7 Bars Carbowax	4	−0.01 ± 0.01 e	10.62 ± 0.37 a
	8	0.00 ± 0.00 e	9.03 ± 0.70 b
−5.2 Bars mannitol	4	0.01 ± 0.01 e	8.60 ± 0.46 b
	8	−0.01 ± 0.01 e	6.82 ± 0.44 c

In plasmolysis the pressure of the protoplast against the wall, even though restrained by the wall from assuming a spherical shape, probably is negligible. Continued shrinkage of plasmolyzed cells with decreasing water potential must result from cell wall dehydration, with concomitant decrease in matric potential rather than in pressure potential. In a soft tissue cells are less distorted by negative matric potential than by negative pressure potential.

The deleterious effects of exposure to isotonic mannitol on subsequent growth could not have been plasmolytically induced. Plasmolysis would have resulted in severe damage (4–7). I could find no published explanation of mannitol damage not involving plasmolysis, and I have no data to warrant speculation.

Carbowax 6000, because it did not penetrate cell walls, came closer than mannitol to mimicking the effect of controlled drying in humid air. The effects of low soil moisture on roots should be more closely duplicated by Carbowax 6000

than by the low molecular weight Carbowaxes, mannitol, or other small solutes. For different reasons, Kaufmann and Eckard (9) reached a similar conclusion.

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