Phytochemistry, 1976, Vol. 15, pp. 1881–1884. Pergamon Press. Printed in England.

EFFECT OF POLLINATION ON CARBOHYDRATE METABOLISM IN YOUNG FRUITS OF *CITRULLUS LANATUS* AND *CAPSICUM ANNUUM*

R. R. WALKER and J. S. HAWKER
C.S.I.R.O., Division of Horticultural Research, G.P.O. Box 350, Adelaide, South Australia, 5001

(*Revised received* 9 *June* 1976)

Key Word Index—*Citrullus lanatus*; watermelon; *Capsicum annuum*; pepper; pollination; fertilisation; parthenocarpy; β-fructofuranosidase; sucrose synthase; sucrose hydrolysis; reducing sugars; starch.

Abstract—During a 9 day period after anthesis the concentration of reducing sugars showed a 6-fold increase in fruits of *Citrullus lanatus*, and a 2-fold increase in those of *Capsicum annuum*. These increases were associated with acid invertase, the specific activity of which was high in ovaries at anthesis and which increased 5-fold in watermelon and 1.5-fold in pepper during the same period. Sucrose synthase apparently plays only a minor role in sucrose hydrolysis. Changes in sugar concentrations and both acid invertase and sucrose synthase activities were similar in fruits developed both after pollination or hormone (NAA) treatment of ovaries. In non-pollinated ovaries of watermelon there was also an increase in invertase activity up to 6 days after anthesis which paralleled the increase in activity in seeded and parthenocarpic fruits. However, there was no increase in either reducing sugars or sucrose, indicating that sucrose is not imported into non-pollinated ovaries. Utilisation of reserve starch may help prolong the life of non-pollinated ovaries for up to one week after anthesis.

INTRODUCTION

Developing fruits are powerful sinks for assimilate [1–3] and it is well established that mobilisation of assimilate to fruits is associated with growth regulator levels in the fruit [3,4]. Watermelon (*Citrullus lanatus*) and pepper (*Capsicum annuum*) ovaries will abort within several days of anthesis if pollination is prevented, or unless growth regulators such as NAA [5] are applied. Such applications partially substitute for pollination and fertilisation and in many plants the resulting parthenocarpic fruits are often indistinguishable from normal fruits in all but fruit shape and seed development [6].

The predominant form in which carbohydrate is translocated in higher plants is sucrose [1], hydrolysis of which yields the reducing sugars, glucose and fructose [7]. Indeed, increased levels of reducing sugars are noticeable soon after pollination in tomato fruits [8]. Either (or both) of the two enzymes, invertase (β-fructofuranosidase) (EC 3.2.1.26) and sucrose synthase (EC 2.4.1.13) may be responsible for cleavage of imported sucrose [7,9–11]. Invertase exists in multiple forms, these being acid and neutral or alkaline forms [7] as well as an insoluble form of the enzyme [12].

Either pollination followed by fertilisation, or hormone treatment may initiate changes in carbohydrate metabolism which determine the pattern of fruit set and development. This investigation covers the period from pollination through fertilisation to the onset of rapid fruit development. It was thought that changes in substrate and enzyme levels accompanying normal fruit development might occur during this period.

RESULTS

During initial development, watermelon fruits exhibited greater increases in fr wt than pepper fruits (Fig. 1). Until Day 6 there was little difference between development of seeded and parthenocarpic fruits of either watermelon or pepper. However, after Day 6 increases in fr wt of parthenocarpic fruits were less than those in seeded fruits of both species. Non-pollinated ovaries of watermelon showed little increase in fr wt (Fig. 1). Furthermore, their growth was not different from that of control ovaries treated with 30% ethanol (minus NAA).

Fruit growth in both watermelons and peppers was accompanied by increases in the level of reducing sugars (Fig. 2). In watermelons, an increase was recorded within 24 hr after pollination or hormone treatment. During the 9 day period after anthesis reducing sugar concentrations increased *ca* 6-fold in watermelon fruits and *ca* 2-fold in pepper fruits. The greater increase in fr wt of watermelons relative to peppers was accompanied by an accumulation of higher concentrations of reducing sugars in watermelons than in peppers. No reducing sugar accumulated in non-pollinated ovaries of watermelon (Fig. 2).

Levels of sucrose in watermelon ovaries at anthesis were less than 0.2% and remained low (<0.2%) in developing fruits. Sucrose concentrations were relatively high in pepper ovaries at anthesis (*ca* 1%) but decreased in developing fruits to less than 0.2% by Day 6 (Fig. 3). This decrease was evident within 24 hr after application of hormone in parthenocarpic fruits, but not until Day 3 in seeded fruits (Fig. 3).

In peppers, starch concentrations increased during the initial 24 hr period after pollination or hormone treatment, but decreased thereafter (Fig. 4). In watermelons, the decrease was less marked, except in non-pollinated ovaries where starch declined from *ca* 0.7% at anthesis to less than 0.1% by Day 6.

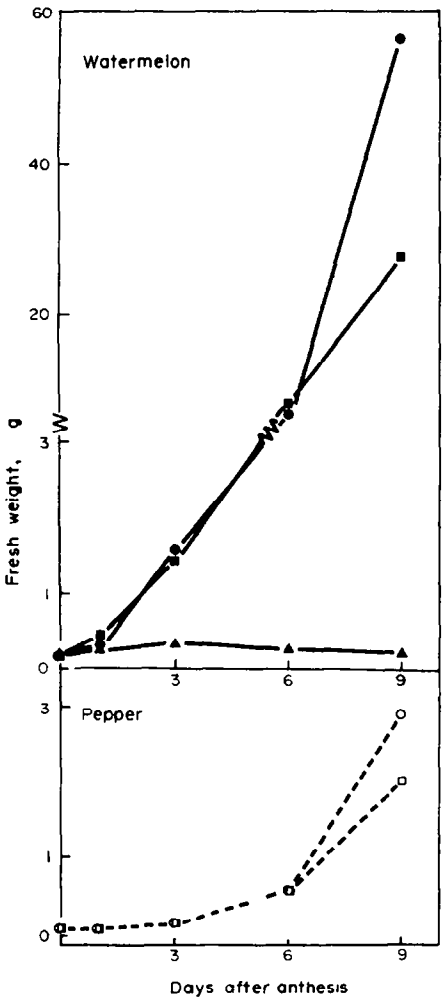


Fig. 1. Growth curves of seeded (●, ○) and parthenocarpic (■, □) fruits of watermelon and pepper and of non-pollinated ovaries (▲) of watermelon.

At anthesis, sp act of both invertase and sucrose synthase were higher in pepper ovaries than in watermelon ovaries. Of the two enzymes, invertase was clearly the most active in both species (Figs. 5, 6).

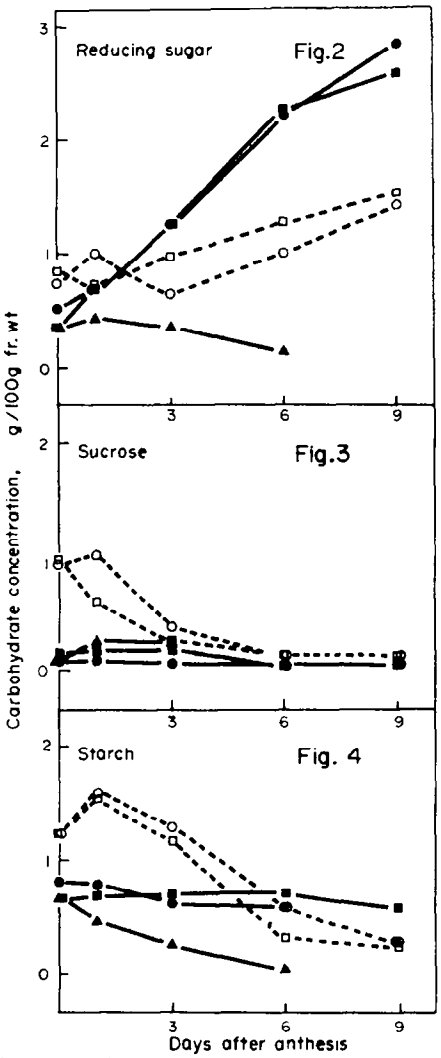
In both watermelons and peppers the sp act of invertase increased after Day 3 (Fig. 5). By Day 9 activities were respectively 5-fold and 1.5-fold higher than activities present in ovaries at anthesis. There was also an increase in sp act of invertase in non-pollinated watermelon ovaries which paralleled the increase in activity recorded in developing fruits but was followed by a decline in activity after Day 6 (Fig. 5). Soluble protein in non-pollinated ovaries remained fairly constant at *ca* 0.5 g/100 g fr. wt. from anthesis to Day 6 post-anthesis, but declined 6-fold between Days 6 and 9.

At Day 6 after anthesis, concentrations of reducing sugars, sucrose and starch and activities of sucrose synthase, soluble and insoluble invertase in non-pollinated watermelon ovaries were not different from corresponding concentrations or activities present in ovaries treated with 30% ethanol (minus NAA).

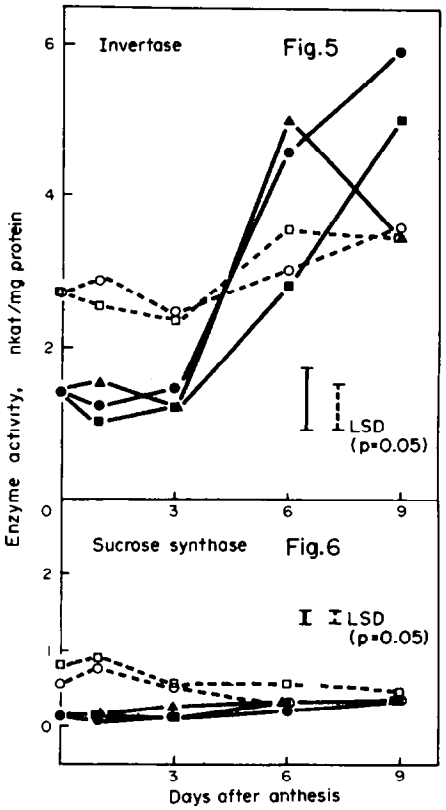
Insoluble invertase was present in fruits of both species during the experimental period. There was a marked decrease in activity of insoluble invertase activity in seeded and parthenocarpic fruits of pepper from Day 0 to Day 9 (Table 1).

Investigation of invertase activity at various pH values from either ovaries at anthesis or 9-day-old fruits of both watermelons and peppers revealed the presence of acid invertase only. Neutral or alkaline invertases were not detected. Soluble invertase in watermelons was active over a wide pH range from about 4 to 6.5 with maximum activity at *ca* 5, while insoluble invertase had a more defined pH optimum at *ca* pH 5. In peppers insoluble invertase was active over a fairly wide pH range from 4 to *ca* 6.5 while soluble invertase was most active from *ca* pH 4 to 5.5.

On a protein basis, greatest activity of soluble invertase in watermelons was located in the inner flesh with smaller activities in the pericarp and ovules (Table 2). On the contrary, expression of activity on a fr wt basis revealed most activity in the pericarp at least in the flesh. Insoluble invertase activity was evenly distributed between pericarp and inner flesh with lesser amounts in the ovules. When enzyme activities were compared with levels of reducing sugars in the various tissues (Table



Figs. 2, 3 & 4. Carbohydrate concentrations in developing seeded (●, ○) and parthenocarpic (■, □) fruits of watermelon (●, ■—●, ■) and pepper (○, □----○, □) and in non-pollinated ovaries (▲) of watermelon.



Figs. 5 & 6. Enzyme activities in developing seeded (●, ○) and parthenocarpic (■, □) fruits of watermelon (●, ■—●, ■) and pepper (○, □----○, □) and in non-pollinated ovaries (▲) of watermelon.

2) it was evident that there was no correlation between invertase activity, soluble or insoluble, and levels of reducing sugars in the various tissues.

DISCUSSION

A correlation existed between increased levels of reducing sugars in seeded and parthenocarpic fruits of watermelon and pepper during a 9 day period after anthesis and increased sp act of acid invertase over the same period. Activity of acid invertase was high in watermelon and pepper ovaries at anthesis and did not appear to be limiting fruit growth. The range of acid invertase activities from anthesis to 9 days post-anthesis was comparable to or higher than activities in radish roots, higher than in carrot roots but lower than in grape berries and tomato fruits [12,17,18]. This invertase appears to be the major enzyme involved in sucrose hydrolysis because the sp act of sucrose synthase in both watermelons and peppers was much lower than that of acid invertase during the experimental period. Activities of insoluble invertase were also high in ovaries and fruits of watermelon and pepper, being comparable to those found in grape berries [12]. However, there was no correlation with the pattern of accumulation of reducing sugars in either watermelon or pepper.

Precautions were taken to avoid formation of invertase–tannin complexes by addition of protective agents which suggests that insoluble invertase of watermelons and peppers is bound to cell walls *in vivo*. There is good evidence that the enzyme is attached to cell walls *in vivo* in aging disks of storage tissues [19], which might explain the increase in insoluble invertase in senescing non-pollinated ovaries of watermelon.

An inverse relationship between acid invertase activity and sucrose content was evident in both watermelons and peppers. Ap Rees [7] has reviewed the occurrence of this relationship in a range of other plant tissues and the evidence which suggests that intracellular acid invertase is located in the vacuole. Sucrose concentration rises in watermelons as their growth rate slows and they approach maturity [20] and this is presumably associated with a decline in acid invertase activity. Location of the enzyme in the vacuole is a likely site for control of absorbed sucrose concentration. The increase in sp act of acid invertase in rapidly expanding watermelon and pepper fruits is probably associated with the increased demand for hexose at that stage of fruit development.

In watermelons, the sp act of invertase was greatest in tissue of the inner flesh. This is the region of greatest cell expansion where cell area increased *ca* 60-fold during

Table 1. Activity of insoluble invertase in non-pollinated ovaries of watermelon and in seeded and parthenocarpic fruits of both watermelon and pepper

Treatment	Watermelon			Pepper	
	Seeded	Parthenocarpic	Non-pollinated	Seeded	Parthenocarpic
Day 0	6.3	4.4	3.5	2.3	3.7
Day 1	6.0	3.2	3.4	2.9	3.9
Day 3	7.7	2.3	3.5	1.7	2.0
Day 6	6.1	2.8	8.2	1.1	1.3
Day 9	4.4	3.5	—	0.6	0.6

Activity = nkat/g fr. wt. (—) = Not measured.

Table 2. Activity of soluble and insoluble invertase and levels of reducing sugars in ovules, pericarp and inner flesh from nine-day-old watermelon fruits

Tissue	Soluble invertase		Insoluble invertase	Reducing sugar
	nkat/g fr. wt	nkat/mg protein	nkat/g fr. wt	g/100 g fr. wt
Ovules	5.5	1.5	4.8	3.1
Pericarp	6.6	4.4	6.0	2.3
Inner Flesh	4.2	7.9	5.8	2.5

the 9 day period (M. Sedgley, personal communication). High sp act of invertase have also been recorded in elongating regions of pea epicotyls [10]. The distribution of invertase activity in fruit tissues was not related to levels of reducing sugars, which suggests differential utilisation of hexose by the various tissues.

Fruit growth and accumulation of reducing sugars are dependent on continued supply of sucrose to developing fruits [1] and sufficient activities of acid invertase for hydrolysis of imported sucrose. The results of this investigation indicated that supply of sucrose rather than acid invertase activity is a major factor limiting any possible increase in ovary size, i.e. non-pollinated ovaries failed to accumulate either reducing sugars or sucrose in spite of an increase in sp act of invertase which paralleled increases in invertase activity in seeded and parthenocarpic fruits.

Absence of starch accumulation in developing fruits of watermelon and pepper was in contrast to the tomato where a marked increase in starch content occurs soon after anthesis [8]. This reflects an inherent difference between the two fruits, i.e. immature tomatoes contain high concentrations of starch [21] while watermelons contain virtually no starch reserve [20]. The fact that non-pollinated ovaries remained alive in the absence of imported sucrose may be associated with the significant decrease in starch concentrations from levels present in ovaries at anthesis, this decrease being much more rapid than that occurring in seeded and parthenocarpic fruits.

Similarities in carbohydrate metabolism between seeded and parthenocarpic fruits indicated that changes induced by hormone application are similar to effects induced by pollination and fertilisation. The possible existence of a threshold concentration of hormone for an effect on carbohydrate metabolism [8] is supported by instances of successful pollination but unsuccessful fertilisation followed by abortion. Obviously, hormone or additional hormone is generated soon after pollination and the threshold may not be exceeded until after fertilisation. In the watermelon, for example, it is possible that hormone produced following pollination results in a mobilisation of sucrose to the ovary, the sucrose being hydrolysed by intracellular acid invertase to form reducing sugars. Continued hormone production, over and above a threshold level, may be required for continued flow of sucrose to the ovary and successful fruit development.

EXPERIMENTAL

Plants of watermelon (*Citrullus lanatus* (Thunb.) cv Sugar Baby) and pepper (*Capsicum annuum* L., cv Long Sweet Yellow) were grown under controlled conditions of 14 hr, 25° day and 10 hr, 20° night. Watermelon flowers were pollinated or treated with hormone in the morning on the day of opening. Pepper flowers were emasculated on the day prior to opening then pollinated or treated with hormone when open. Parthenocarpic fruits were induced using 500 ppm NAA in 30% EtOH applied to the base of the cut style of watermelon flowers and using 5000 ppm NAA in lanolin paste, similarly applied to pepper flowers. These concns of NAA have been found to be optimum for watermelon and pepper (M. Sedgley, personal communication). In watermelon, control ovaries were those treated with only 30% EtOH. Growth curves were constructed using fr wt from a minimum of 10 watermelon ovaries or fruits and 20 pepper ovaries or fruits.

Preparation of enzyme extracts. Several whole ovaries or wedges from several larger fruits were used to prepare each extract. Triplicate extracts were prepared from each treatment. Tissue, 0.5–3.0 g, was ground in a mortar with 10 ml of 0.1 M Tris–HCl buffer, pH 7.5 containing 20 mM EDTA, 11 mM Na diethyldithiocarbamate and 15 mM cysteine–HCl. The brei was centrifuged at 2000 *g* for 10 min. The residue was retained for determination of insoluble invertase activity [12]. The supernatant was brought to 60% saturation with $(NH_4)_2SO_4$, then centrifuged at 12000 *g* for 10 min. The protein ppt was dissolved in 3 ml of 5 mM Tris–HCl, pH 7 and then desalted on a 15 ml column of Sephadex G-25, prewashed with the same buffer. The final extract (2 ml) was used for enzyme assays and soluble protein determinations.

Enzyme assays. Invertase activity was determined at 30° by measuring the appearance of reducing sugar [13] in 0.1 M citrate buffer, pH 5, containing 0.1 M sucrose and enzyme. For determination of invertase activity as a function of pH, 0.1 M citrate–Pi or 0.1 M Pi buffers were used. Sucrose synthase was assayed at 30° by following the incorporation of fructose-[$U^{14}C$] into sucrose [12] in the presence of 250 mM sucrose and at pH 8.5 to overcome the interference caused by invertase. Proteins were assayed using the method of ref. [14] with BSA as standard.

Carbohydrate determinations. Several whole ovaries or wedges from several larger fruits were used to prepare each extract. Triplicate extracts were prepared from each treatment. Tissue, (0.5–3.0 g) was boiled in 70% EtOH for 3 min and then ground in a glass piston homogeniser. After cooling, the suspension was centrifuged at 12000 *g* for 10 min. The supernatant was decanted and made to 20 ml and the ppt was set aside for starch determination as described previously [15]. Aliquots from the supernatant extracts were assayed for reducing sugars [13] and sucrose. Sucrose was determined by measuring keto sugars (including sucrose) [16] in extracts which had been treated for 10 min at 100° with 0.2 N NaOH both with and without prior invertase treatment.

Acknowledgements—Technical assistance by J. Milln and J. Valentine is gratefully acknowledged.

REFERENCES

1. Wardlaw, I. F. (1968) *Bot. Rev.* **34,** 79.
2. Mullins, M. G. (1967) *Australian J. Biol. Sci.* **20,** 1141.
3. Crane, J. C. (1964) *Ann. Rev. Plant Physiol.* **15,** 303.
4. Crane, J. C. (1969) *Hort. Science* **4,** 108.
5. Wong, C. Y. (1938) *Proc. Am. Soc. Hort. Sci.* **36,** 632.
6. Wong, C. Y. (1941) *Bot. Gaz.* **103,** 64.
7. ap Rees, T. (1974) In *MTP International Review of Science, Plant Biochemistry*, Vol. II, p. 89, Ed. D. H. Northcote, Butterworths, London.
8. Marrè, E. and Murneek, A. E. (1952) *Plant Physiol.* **28,** 255.
9. Hawker, J. S. (1971) *Phytochemistry* **10,** 2313.
10. Maclachlan, G. A., Datko, A. H., Rollit, J. and Stokes, E. (1970) *Phytochemistry* **9,** 1023.
11. Wolosiuk, R. A. and Pontis, H. G. (1974) *Molec. Cell. Biochem.* **4,** 115.
12. Hawker, J. S. (1969) *Phytochemistry* **8,** 337.
13. Nelson, N. (1944) *J. Biol. Chem.* **153,** 375.
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265.
15. Hawker, J. S., Marschner, H. and Downton, W. J. S. (1974) *Australian J. Plant Physiol.* **1,** 491.
16. Ashwell, G. (1957) *Methods in Enzymology* Vol. 3, p. 75. Academic Press, New York.
17. Ricardo, C. P. P. and Sovia, D. (1974) *Planta* **118,** 43.
18. Manning, K. and Maw, G. A. (1975) *Phytochemistry* **14,** 1965.
19. Little, G. and Edelman, J. (1973) *Phytochemistry* **12,** 67.
20. Pratt, H. K. (1971) In *The Biochemistry of Fruits and their Products* Vol. 2, p. 207, (Hulme, A. C. ed.). Academic Press, New York.
21. Hobson, G. E. and Davies, J. N. (1971) In *The Biochemistry of Fruits and their Products* Vol. 2, p. 437, (Hulme, A. C., ed.). Academic Press, New York.