FINAL REPORT

Covering Period: June 1, 1989 to May 31, 1993

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POST-HARVEST TECHNOLOGY OF SOME UNDER-EXPLOITED TROPICAL FRUIT

Principal Investigator: C. M. Sean Carrington
Grantee Institution: University of the West Indies (Barbados)

Collaborator: Dr. Donald J. Huber
Institution: University of Florida (Gainesville)

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EXECUTIVE SUMMARY

The purpose of the project was to characterise the development of two promising tropical fruit, breadfruit (*Artocarpus altilis*) and soursop (*Annona muricata*), and investigate procedures for improving their post-harvest life.

It was shown that development of soursop fruit commences after a variable 'rest' period of the blossom, the fruit then taking some 4 months to reach maturity. Reliable characteristics for use in recognising fruit maturity (maturity indices) were identified and are now being used by commercial growers in Grenada. Preliminary storage studies revealed refrigeration at 14°C with film wrapping extended shelf life of soursop fruit by over a week.

Breadfruit were found to reach maturity 15-19 weeks after the female inflorescence was detectable and maturity indices for optimal harvesting were identified. While it was known that the fruit had a high respiratory rate, its low rate of production of the ripening hormone, ethylene, came as a surprise. In keeping with this, the fruit, especially the more mature, were found to be quite sensitive to ethylene exposure, a finding of some practicality. Storage at 13°C delayed fruit softening by 10-14 days but the fruit skin rapidly turned an unsightly brown. Plastic film wrapping of the fruit minimised this discoloration and, of some ten plastic films investigated, High Density Polyethylene (HDPE) 40 μm film maintained fruit of acceptable eating quality, as judged by a taste panel, even after 2 weeks' refrigerated storage. Trial shipments to Europe by air freight supported this approach. These handling techniques are now used commercially for breadfruit export from the Caribbean. This project has equipped the host institution with the instrumentation and scientific expertise to tackle future research on tropical fruit commercialisation.

RESEARCH OBJECTIVES

Breadfruit and soursop are two promising export fruit crops for the Caribbean but which required scientific study for effective exploitation. An understanding of their development was lacking as were techniques for extending their post harvest life. The first project objective was to characterise the development of these fruit so as to devise maturity indices for optimal harvest. The second objective was to characterise the ripening process in both fruit with a view to delaying and manipulating this. The final aim of this study was to examine handling procedures such as hydrocooling, refrigerated storage, fruit coating, film wrapping and ethylene scrubbing so as to maintain fruit for extended periods without losses due to ripening and spoilage.

These fruit fit the category of minor fruit in terms of the scale of cultivation compared to major fruit such as banana, pineapple and mango. Expansion of this minor fruit industry is a major plank of agricultural diversification in the Eastern Caribbean and this project provides baseline information for such a programme. Our work on soursop has complimented that of Ms. Sintra Persad at the Caribbean Agricultural Research Development Institute (CARDI) in improving soursop production in Grenada while the breadfruit work has been useful for shippers throughout the region and has also been of interest to Dr. Diane Ragone and her colleagues at the National Tropical Botanical Garden, Hawaii, in their programme of cultivar selection (Ragone, 1989). The innovative aspect of the project is purely in the two fruit being studied, especially in evaluating post harvest techniques which have proven effective with temperate fruit. The University of the West Indies utilised the graduate student working on this project as a trainer in their short Continuing Education Programme in Agricultural Technology (CEPAT) courses and this served to disseminate practical results from the project as they became available.

METHODS & RESULTS

Characterisation of soursop development & ripening

This work has been published and can be consulted in the two papers (Worrell & Carrington, 1993; Worrell *et al.*, 1994) in the Appendix.

Characterisation of breadfruit development & ripening

Methods

Equatorial and polar diameter measurements were made weekly on 12 developing fruit of breadfruit (*Artocarpus altilis* Fosk., unnamed white-fleshed cultivar) using a ruler. At fortnightly intervals, over 5 months, a replicate of 6 fruit of known age were harvested, weighed to determine fresh weight, halved polarly, then frozen and stored at -20°C. One half of each fruit was dried at 120 °C for 72 h to determine dry weight. Using a 7 mm diameter cork borer, 10

tissue discs, including the fruit peel, were prepared from the frozen fruit of various ages and chlorophyll quantified by the method of Porra *et al.* (1989). Further flesh samples (2 g) were homogenised in water using a Polytron, centrifuged (800 *g*; 10 mins.) and the supernatant analysed for reducing and non-reducing sugars (Chaplin, 1986). Starch levels were assayed by preparing alcohol insoluble solids from frozen fruit samples, treating 2 mg portions of these (quadruplicate samples per fruit stage) with 20 units of amyloglucosidase (ex *Rhizopus niveus*, Seikagaku Kogyo, Japan) in acetate buffer (0.5M; pH 4.8; 0.1% NaN_3) for 24 h at 45°C and measuring the resulting reducing sugars (Chaplin, 1986).

Early mature breadfruit (first signs of surface latex and slightly domed polygonal segments) and late mature breadfruit (surface latex, relatively flattened polygonal segments without prominent spur at their centre) were harvested using a custom-built harvester with bag on the end (Satney, 1992). Fruit were placed singly in 25 L plastic chambers in a flow-though system, ventilated (~1.5 L/min.) with air which first passed through a saturated $KMnO_4$ solution. CO_2 and C_2H_4 were measured in the exit lines with an infra-red gas analyser (Model 225-MkIII; ADC Ltd., Hoddesdon, U.K.) and a gas chromatograph (Model 10S; Photovac, New York, U.S.A.) respectively. Fruit texture was assessed by finger feel on a 3 point scale (1 = firm, 2 = can be reversibly deformed, 3 = soft). Daily starch and sugar levels were determined during ambient storage as described above.

Results

Fig. 1 depicts changes in gross dimensions and starch content of developing breadfruit. Fruit reach full size about 14 weeks after first detectable, the pattern being a simple sigmoid curve (Fig. 1A). In contrast, from a weight perspective, growth is double sigmoid (Fig. 1B) with the bulk of weight gain in the latter phase of growth. Starch accumulation is a major contributor to this (Fig. 1C) such that the first growth phase can be regarded as a size generation phase and the second, the period of laying down of starch reserves. Consistent with this sugar levels decline markedly by the time fruit are 8 weeks old, suggestive of such sugars being diverted into starch production (Fig. 2).

Breadfruit do not undergo a marked colour change during development, as do other fruit. There is a slight darkening of the green skin, followed by a slight paling as maturity is reached. This is suggested by chlorophyll measurements (Fig.3) although these trends are not statistically significant. Taste tests were carried out on fruit of age 11-21 weeks and revealed that fruit 15-19 weeks old were acceptable to the consumer (data not shown). Such fruit were characterised by;- (1) surface latex, (2) relatively flattened polygonal segments & (3) lack of a prominent central spur in each polygon. These were adopted as the maturity indices for breadfruit.

It has already been noted that breadfruit is a climacteric fruit with one of the highest rates of CO_2 production (Biale & Barcus, 1970). Our work confirms this observation (Fig. 4A) but, interestingly, the peak C_2H_4 production during the climacteric (1.6 µL/kg/h) is surprisingly low. Fruit softening was coincident with the climacteric rise in both CO_2 and C_2H_4 (Fig. 4C). Starch levels declined

Figure 1 Changes in size (A), fresh and dry weight (B), and starch (C) in developing breadfruit (mean of 6 fruit ± SD).

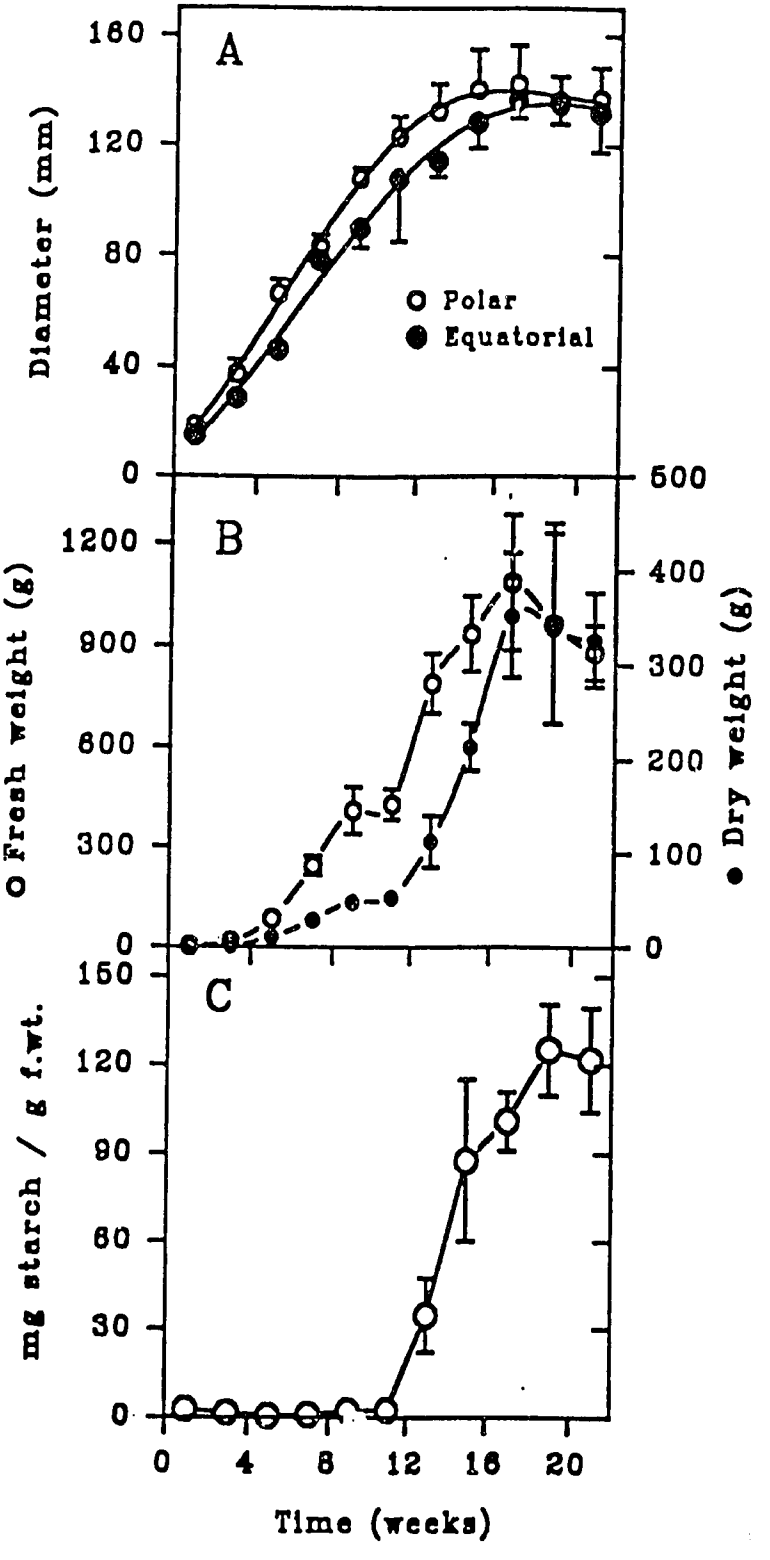


Figure 2 Reducing sugars (open bars) and total sugars (shaded bars) in breadfruit during development (mean of 6 fruit ± SD).

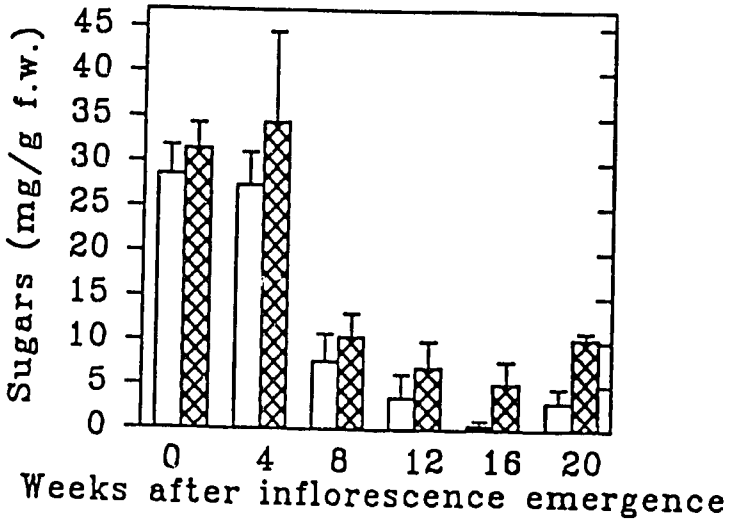


Figure 3 Chlorophyll in breadfruit peel during development (mean of 6 fruit ± SD).

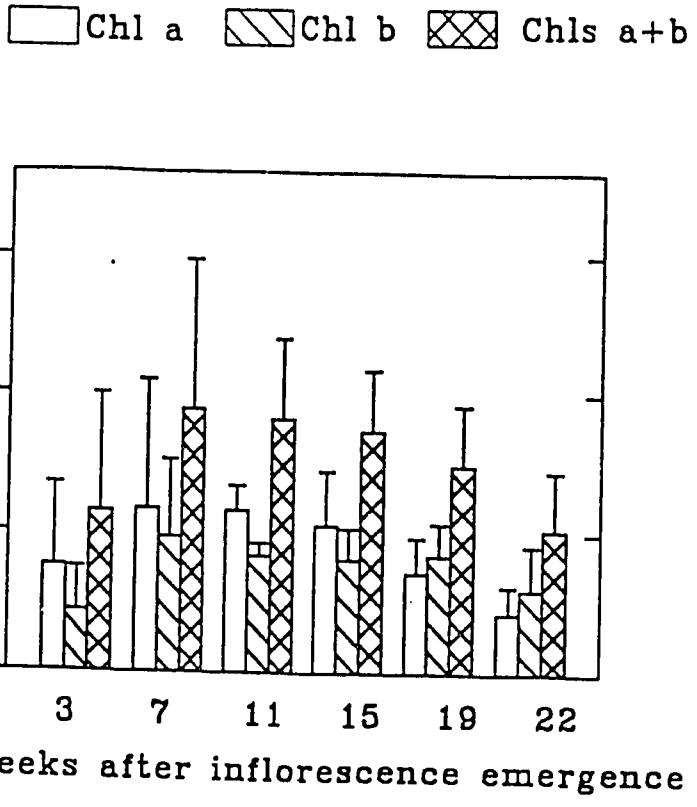
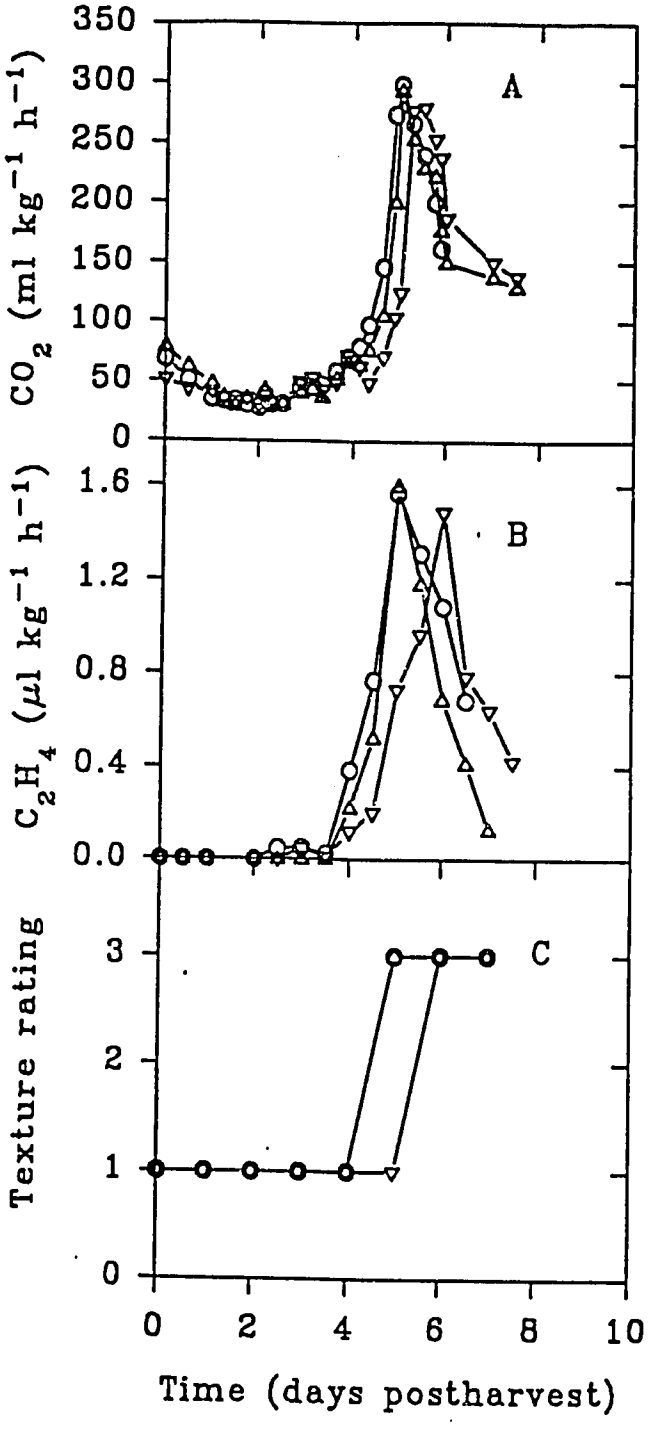


Figure 4 Respiration (A), ethylene production (B), and texture in 3 early mature breadfruit during storage at ambient.



abruptly to about ½ the initial value after 4 days of ambient storage. This more or less coincided with the climacteric and was also characterised by an 8X rise in total sugars (data not shown).

Refrigerated storage of breadfruit in coatings & films

Methods

Replicate batches of 8 fruit were stored at ambient (25-30°C) or in refrigerated incubators at 7, 12, 13, 14, 15, 16, 17, 22, 25, 30 and 35°C, weighed and rated daily for texture by finger feel. Ethylene and CO_2 production were monitored in 6 fruit individually, as described previously, except that fruit were held at 13°C in a refrigerated incubator.

Three replicates of 10 fruit each per treatment were brush coated with 1.5 % Semperfresh F (Semper Biotechnology Ltd., Reading, U.K.), 3 % Nutrisave (Nova Chem Ltd., Nova Scotia, Canada), full strength Sta-Fresh MP (FMC Corporation Lakeland, FL, U.S.A.), 1.5 % chitosan (Sigma, St. Lous, MO, U.S.A.) or left uncoated. Fruit were stored at ambient and monitored every 2 days for weight loss, softening, skin discoloration and microbial growth. The experiment was repeated at 13°C.

A needle with attached 5 ml hypodermic syringe was stuck into each of 36 breadfruit and silicone dental impression material applied at the needle-fruit interface to give an air-tight seal. The plunger was removed from each syringe which was then capped by a self-sealing rubber septum. One batch of six fruit was left uncoated while one of the coatings described above was applied to each of the remaining 5 batches of fruit. Fruit were stored at ambient and gas samples were removed daily from each syringe chamber for CO_2 and C_2H_4 analysis (as previously described). Oxygen was also analysed using a Protox oxygen meter (Gow-Max Instrument Co., Gillingham, Kent, U.K.). The experiment was also repeated with fruit storage at 13°C.

Batches of 10 freshly harvested breadfruit were individually sealed in each of the following plastic films using an heat sealer;- high density polyethylene (HDPE) of 25, 40 and 60 μm thickness, low density polyethylene (LDPE) of 30, 40 and 60 μm thickness (Superior Plastics, Barbados), EHC 15 and 60 μm and LLP 60 μm Clysar shrink films (Dupont, Delaware, U.S.A.) and SealedFresh 1.5 mil (SealedFresh Inc., Denver, CO, U.S.A.). Weight loss, softening, discoloration and microbial growth were monitored daily during subsequent storage at ambient. The experiment was repeated twice and then three times but with storage at 13°C.

Thirty-three fruit were prepared for internal gas sampling as described above, one batch of 3 fruit being left unbagged and the remainder sealed in the 10 film types listed previously, 3 fruit per film. Fruit were held at ambient and CO_2, C_2H_4 and O_2 levels were measured daily as described above. This experiment was repeated with storage at 13°C.

Batches of 10 fruit, unwrapped or sealed in one of the films listed above were stored at 13°C for 1, 2 or 3 weeks. At the end of each storage period, cooked samples of 2 fruit per treatment, as well as cooked samples of freshly harvested fruit, were given to an untrained taste panel for appraisal.

Figure 5 Respiration (A, B), C_2H_4 production (C, D) and texture (E, F) of early mature breadfruit stored at 13°C. Each of 6 fruit is represented by a different symbol.

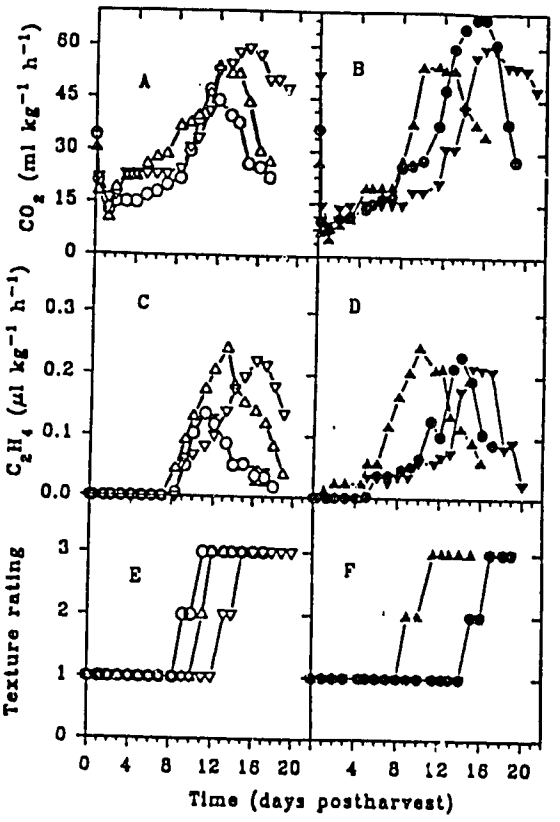
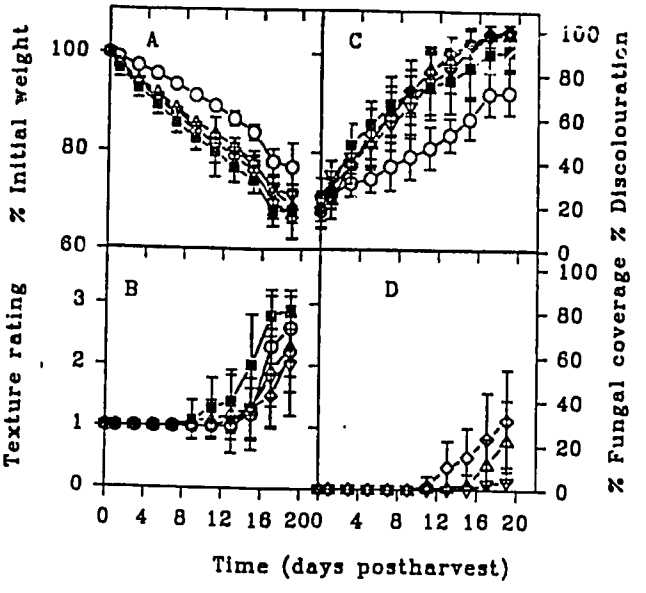


Figure 6 Weight loss (A), texture (B), discoloration (C) & fungal coverage (D) in breadfruit stored at 13°C with one of 4 coatings or left uncoated (mean of 10 fruit ± SD).

Δ Semperfresh ◊ Nutrisave
∇ Chitosan o Stafresh
■ unwrapped



Batches of 4 early mature and late mature (as previously defined, p.5) fruit were enclosed in air-tight 25 L chambers and exposed to C_2H_4 at 5, 50 or 500 ppm for 6 or 24 h at ambient. Gas mixtures were provided by dilution of a 99 % C_2H_4 standard (TriGas Inc., Jupiter, FL, U.S.A.) and final concentration confirmed by GC. Fruit were then stored at 13°C and monitored every 2 days for textural changes and skin discoloration.

Freshly harvested early mature fruit were either left unbagged or sealed in one of the several films previously listed, 20 fruit per treatment. For each film treatment, a 9 g sachet of $KMnO_4$-coated silica beads (Ethylene Control Inc., Selma, CA, U.S.A.) was enclosed within the film of half of each batch of fruit. Fruit were stored at ambient and monitored for softening and discoloration. The experiment was repeated with storage at 13°C.

Results

Over a 12 day storage period, fruit stored at 7, 12 and 13 °C showed no softening while fruit at higher temperatures softened rapidly, the higher the temperature the faster the rate (data not shown). Weight loss was as low as 15% in 1 week at the lower temperatures and as high as 15% in 3 days at ambient. Fruit at 7°C showed some signs of chilling injury and so 13°C was used as the optimal storage temperature in all subsequent experiments. It typically took 8 h to air-cool breadfruit to the 13°C holding temperature and while this could be reduced to half or a quarter the time by hydrocooling fruit in 13°C water or an ice-water mixture respectively, experiments showed these shortened cooling times did not enhance post-harvest life (data not shown). The extended storage life of fruit at 13°C is readily understood when respiratory behaviour at this temperature was examined. The climacteric, as evidenced by both peak CO_2 and C_2H_4 production, was delayed by about 10 days and was of much less magnitude (Fig. 5). Fruit softening was similarly delayed (Fig. 5E & F). One drawback of such refrigerated storage was the unsightly browning of the fruit skin which occurred within 1-2 days of low temperature storage. This could be alleviated by storing fruit submerged in water, suggesting it was a water loss problem. Attempts were made to address this by both applying coatings to fruit and sealing fruit in plastic films.

Coatings did tend to delay the onset of softening at ambient by 2-3 days but no coating performed consistently better than the other (data not shown). At 13°C, coatings did little to consistently improve keeping quality but did ameliorate the skin discoloration problem, Stafresh being particularly effective in this regard (Fig. 6C). Such coated fruit, both at ambient and 13°C, tended to show depressed O_2 and elevated CO_2 levels (data not shown) but there was great inconsistency in the results suggesting a lack of reproducibility in the application of the coatings or leaky air-needle junctions. Other parameters such as starch and sugar levels in these coated fruit were examined but the general poor effect of the coatings on keeping quality led to a shift of interest to the effects of film-wrapping.

The most consistent effect of film wrapping was to dramatically reduce water loss to about 3 % after 3 weeks' storage vs. 20 % in 1 week for unwrapped fruit. Softening was generally delayed by 1 week, the thicker films maintaining

texture better (data not shown). Skin colour was conserved by film wrapping, especially by the thinner films over the longer time frame. In two experiments certain thin films and the SealedFresh film maintained fruit in perfect condition for 2-3 weeks. Attempts to replicate these results were unsuccessful. It seemed possible the success of these ambient experiments might have been related to low night temperatures at that time of year but repeat experiments in refrigerated incubators with suitable temperatures never gave similar results.

While it was possible that fruit water status or an altered physiological state of the fruit could play a role in maintaining fruit quality in film wrapping at ambient this would have been a major study in itself and so the focus shifted to film wrapping as an adjunct to refrigerated storage.

Film wrapping at 13°C drastically reduced water loss and skin discoloration and in most films fruit did not soften even after 21 days. Fig. 7 shows typical results for only 4 films but there was little or no difference between the full set of films. As Fig. 8 shows, film wrapping led to a dramatic drop in $[O_2]$ in fruit and elevation of $[CO_2]$ as storage time progressed. CO_2 levels were higher with thicker films but this did not effect the softening behaviour of fruit. C_2H_4 levels rose during storage b : this pattern was not significantly different between films (Fig. 8E). Since all films seemed equally good at maintaining fruit firmness at 13°C it was important to establish the eating quality of such fruit.

Table 1 shows a summary of the sensory characteristics as assessed by a taste panel of cooked breadfruit which had been stored film-wrapped for 2 weeks at 13°C. After 1 week of storage, fruit in the LDPE and HDPE films were acceptable though inferior to freshly harvested fruit, a slight sweetening being generally noticed (data not shown). By the second week only the HDPE 40 μm film maintained acceptable quality, with off-flavours, sweetness and soft texture making all other treatments unacceptable (Table 1). By 3 weeks storage none of the film wrapped fruit were acceptable (data not shown). Trial shipments to Holland by air using fruit in cardboard boxes with or without individual HDPE 40 μm film-wrapping showed this treatment was commercially applicable with spoilage greatly reduced (data not shown). Further experiments were carried out to devise a recommended retail handling procedure in which film-wrapped fruit were stored at 13°C for 1 or 2 weeks and then transferred to ambient and either unbagged or left film-wrapped. These results clearly showed that fruit should be left bagged until used (data not shown).

The low C_2H_4 production rates reported above suggested that the fruit might be quite C_2H_4-sensitive. Accordingly, this was investigated as well as the usefulness of C_2H_4-scrubbers. Fig. 9 shows that the early mature fruit are relatively insensitive to C_2H_4 in terms of accelerating softening. On the other hand, prolonged (24 h) exposure to even the lowest concentration of C_2H_4 used (5 ppm) promoted skin browning relative to the control fruit (Fig. 9D). Late mature fruit, however, tended to soften faster even after a 6 h exposure to 50 ppm C_2H_4 (data not shown) emphasising the importance of using early mature fruit for export markets. Fig. 10 shows the effect of including an C_2H_4 scrubber ($KMnO_4$-containing sachet) with film-wrapped fruit stored at ambient. There seems little effect on softening (Figs. 10A & B) but there might be a significant effect with some films of the scrubber in retarding skin browning (Figs. 10C &

Figure 7 Weight loss (A), texture (B), discoloration (C) & fungal coverage (D) in breadfruit stored at 13°C in 4 films or unwrapped (mean of 10 fruit ± SD).

Δ LLP 60 µm ◊ SealedFresh ▽ EHC 60 µm
o EHC 15 µm ■ unwrapped

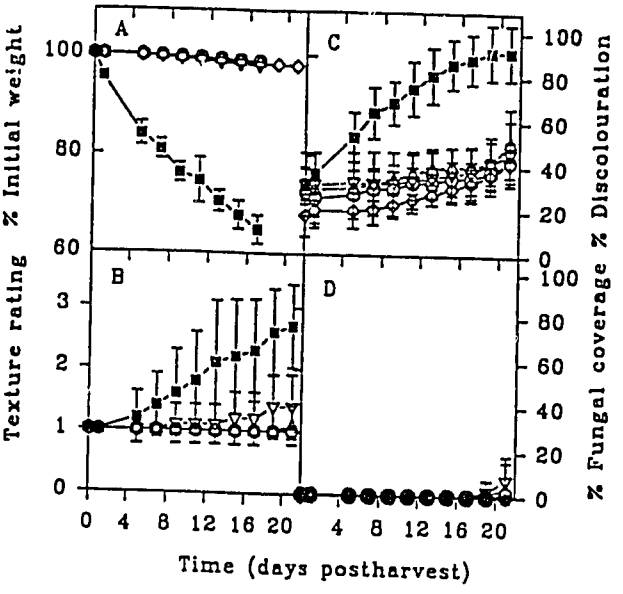


Figure 8 Texture (A), discoloration (B) and concentrations of O_2 (C), CO_2 (D) and C_2H_4 (E) of breadfruit stored in films at 13°C (mean of 3 fruit).

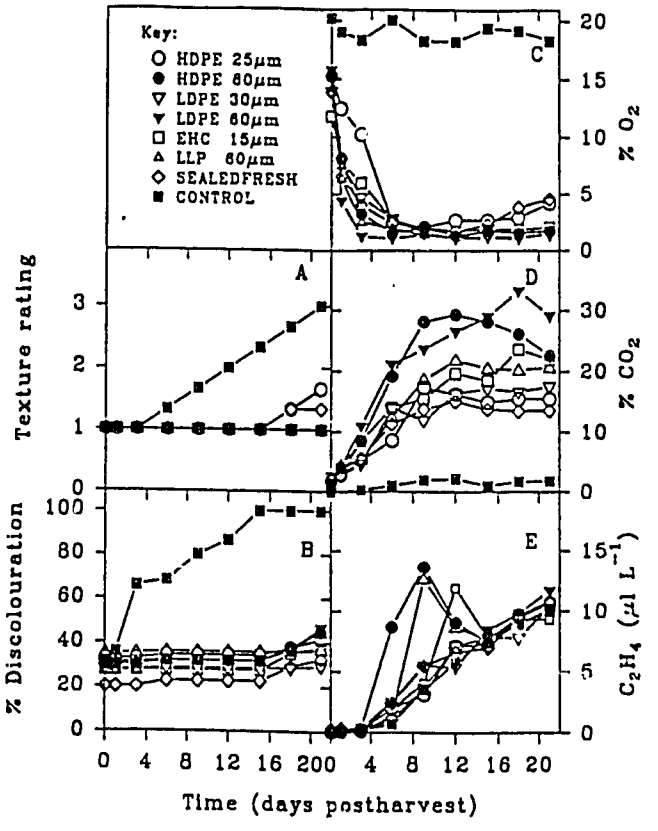


Table 1 Sensory characteristics of breadfruit after 2 weeks' storage at 13°C in film wrapping. Statistical analysis by ANOVA, with separation of treatment means by Tukey's Honestly Significant Difference Test (α=0.05). Data in each column followed by the same letter are not significantly different from each other.

Films	SENSORY QUALITY				
	Palatability	Flavour	Sweetness	Texture	Discolour-ation
LDPE 30μm	1.13 bc	1.60 cd	4.27a	5.33 bc	1.60 f
LDPE 40μm	1.20 bc	2.07 cd	3.57abc	5.76 b	1.87 f
LDPE 60μm	1.73 b	2.33 c	3.80ab	5.63 b	2.47 d ef
HDPE 25μm	2.67a	3.47ab	2.60 cd	4.60 cd	7.27a
HDPE 40μm	3.00a	3.20 b	3.20abcd	4.20 d	5.33 bc
HDPE 60μm	3.33a	4.00a	4.07a	3.87 d	6.40ab
EHC 15μm	1.42 bc	1.92 cd	2.71 cd	5.58 b	3.83 cde
EHC 60μm	1.17 bc	1.75 cd	2.17 d	7.12a	2.78 def
LLP 60μm	1.25 bc	2.08 cd	2.83 bcd	7.25a	4.00 cd
Sealed Fresh	1.00 c	1.42 d	2.42 d	6.84a	2.25 ef
Fresh	6.25	6.81	2.70	4.67	8.35

	Palatability	Flavour	Sweetness	Texture	Discoloration
1	inedible	strong off flavour	not sweet	too soft	extremely discoloured
3	just edible	slight off flavour	slightly sweet	slightly soft	very discoloured
5	good	just pleasant	sweet	firm	slightly discoloured
7	very good	pleasant	very sweet	too firm	v. slightly discoloured
9	excellent	very pleasant	extremely sweet	much too firm	not discoloured

Figure 9 Effects of 6 h (A, C) and 24 h (B, D) exposure of early mature breadfruit to C_2H_4 on subsequent post-harvest life at 13°C.
o 5 ppm, ▽ 50 ppm, △ 500 ppm, ■ control

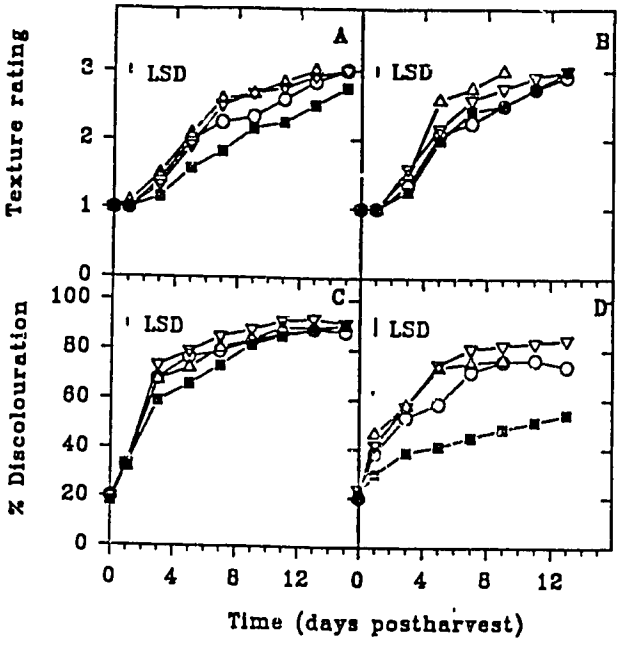
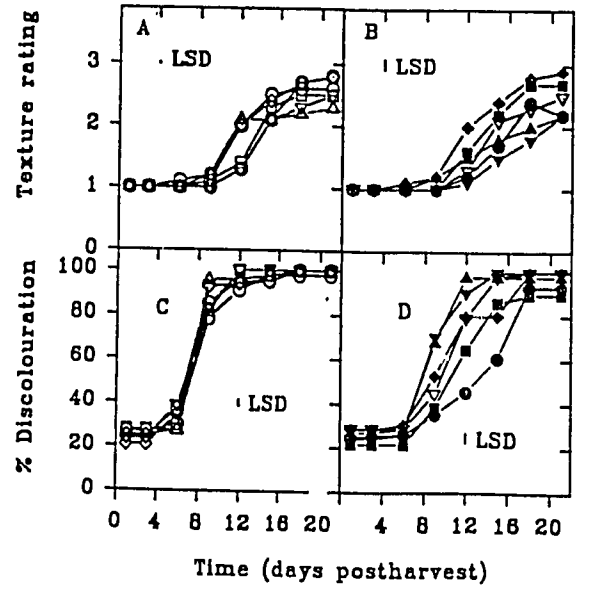


Figure 10 Texture and discoloration of breadfruit stored in films at ambient without (A, C) or with (B, D) C_2H_4-scrubbers.
Without C_2H_4-scrubbers:
o HDPE 25, ▽ HDPE 60, □ LDPE 30, △ LDPE 60, ◇ EHC 15, ⊙ LLP 60
With C_2H_4-scrubbers:
● HDPE 25, ▼ HDPE 60, ■ LDPE 30, ▲ LDPE 60, ◆ EHC 15, ▽ LLP 60



10D). A similar effect was also noted with storage at 13°C (data not shown). These promising results notwithstanding, the modest improvement provided by the scrubber must be weighed against its price, $ US 0.14, the wholesale price of a single breadfruit (both 1992 prices)!

IMPACT RELEVANCE & TECHNOLOGY TRANSFER

The project has resulted in a shift in research interest for the Principal Investigator (PI) to that of post harvest biology of tropical fruit. The timing of this could not have been better with a new regional fruit production thrust, incorporating the launching of a networking group in the Caribbean on tropical fruit and a journal, *Tropical Fruits Newsletter*, all initiated by the Inter-American Institute for Co-operation in Agriculture (IICA). The Biology Department of the University of the West Indies in Barbados, as a result of this project, is now fully equipped to tackle this type of useful research. The graduate student trained through this project has submitted his PhD thesis and plans to continue to work in the Caribbean as a post-harvest scientist after a further research stint as a post-doctoral fellow. The US collaborator has also benefited from an increased appreciation of tropical fruit biology. CATCO and FINTRAC, the commercial shipping companies with which we collaborated, utilised results of this study in their own shipping of breadfruit. Our maturity indices developed for soursop are now being used in the region, especially in Grenada where we helped growers through CARDI.

PROJECT ACTIVITIES / OUTPUTS

Meetings Attended:

Graduate student & PI - 87th Meeting of the American Society for Horticultural Science, Tuscon, AZ, November 4-8, 1990.
PI - International Conference on the Development of New Crops, Jerusalem, March 8-12, 1992
PI - USAID/BOSTID Networking Meeting on Tree Crops, Costa Rica, April 2-8, 1992.
Graduate student & PI. - 10th Annual Conference of the Barbados Society of Technologists in Agriculture, November 11 & 13, 1992.
PI - 29th Annual Meeting of the Caribbean Food Crops Society, Martinique, July 4-10, 1993.
PI - 3rd Regional Workshop on Tropical Fruit Crops, Grenada, May 16-20, 1994.

Graduate student - Instructor on several Continuing Education Programme in Agricultural Technology (CEPAT) Courses:

"Improved post-harvest technologies for tropical fruits and vegetables"
Dominica, July 1-5, 1991.
Grenada, November 15-20, 1992.
St. Vincent, November 29 - December 3, 1993.

"Export marketing of fresh agricultural produce"
Barbados, July 3-15, 1994.

Training:

Graduate student attended an intensive "Short Post-Harvest Physiology Course" run by the University of California, Davis, June 17-25, 1990.

Publications:

Worrell, D.B. & Carrington, C.M.S. (1993) "The waiting game of soursop" *Tropical Fruits Newsletter* (6) 8.

Worrell, D.B., Carrington, C.M.S. & Huber, D.J. (1994) "Growth, maturation and ripening of soursop (*Annona muricata* L.) fruit" *Scientia Horticulturae* 57 7-15.

Worrell, D.B. (1994) The Breadfruit: a study of fruit development and post harvest storage, PhD Thesis, UWI, Barbados, 329 pp.

Conference Proceedings:

Worrell, D.B., Carrington, C.M.S. & Huber, D.J. (1990) "Characterisation of fruit development and ripening in soursop and breadfruit." HortScience 25 147.

Carrington, C.M.S. & Worrell, D.B. (1992) " Development and storage of breadfruit: a recent export crop of the Caribbean" Abstracts of the Conference on the Development of New Crops, Jerusalem, 68.

Carrington, C.M.S. & Worrell, D.B. (1992) " Development and storage of breadfruit: a recent export crop of the Caribbean" Abstracts of the Tree Crops Networking Meeting, Costa Rica.

Worrell, D.B. & Carrington, C.M.S. (1992) " Development and storage of breadfruit: a recent export crop for Barbados" Proceedings of the 10th Annual Conference of the Barbados Society of Technologists in Agriculture, 28-31.

PROJECT PRODUCTIVITY

The project achieved most but not all of its goals. The characterisation of fruit development with identification of maturity indices was completed for both breadfruit and soursop. Likewise, the ripening process in these two fruit was also fully investigated. In the case of soursop, this work has since been published (Worrell & Carrington, 1993; Worrell *et al.*, 1994). The major objective not achieved was the development of a post harvest handling procedure for soursop. This work was initiated, with some form of film wrapping as an adjunct to refrigerated storage at 14°C being particularly most promising. These studies were abandoned, however, as it became clear that the project was over ambitious in trying to proceed to study two different fruit in the detail required. This was especially a problem for the graduate student. The initial characterisation work on the two fruit was enough to constitute an MPhil. thesis. It was clear that the student had the potential to upgrade to PhD registration but this would require focusing on only one fruit for the final phase of the project. The decision was made to stick to breadfruit as this was the more economically important of the two. In addition, the Controlled Atmosphere Storage (CAS) experiments originally envisaged were not carried out as we became aware of studies which had already been carried out in Trinidad on breadfruit using this approach (Maharaj & Sankat, 1990; Ramlochan, 1991). The graduate student on this project, Desmond Worrell, has submitted his PhD thesis and at the time of writing is awaiting his *viva* examination. It should be possible to publish 3 or 4 papers from this thesis. In terms of training of the PI and graduate student, the project fulfilled its objective.

FUTURE WORK

Two aspects of the project deserve further investigation. It would be useful to undertake further work on the refrigerated storage of film-wrapped soursop. In view of the plant disease problems we encountered in our preliminary work a plant pathologist would need to be involved. Ms. Sintra Persad, Plant Pathologist, CARDI, Grenada, has expressed a willingness to collaborate on this and the ready availability of soursop in Grenada (relative to Barbados) makes this an attractive possibility, if we can get funding. Secondly, the success achieved at one stage with ambient storage of film-wrapped breadfruit requires further study. I propose to tackle this by altering the water status of the fruit (e.g. by imbibition, air-drying) prior to bagging and by looking carefully at different maturities.

Since completing this project I have submitted a joint proposal to the European Community for development of a transgenic banana deficient in ethylene synthesis. This bid was unsuccessful but the project received an "A" rating. I have also submitted a CDR proposal to AID on Barbados Cherry and was invited to submit a full proposal. I have not done this but did carry out a mini-project funded by IICA on this crop. The serious bird damage problem I encountered with this has dissuaded me from further work on this crop. I have

also been looking at the biochemistry of softening in avocado (Carrington, 1994) and have spent two stints in the U.S.A. (UNESCO Fellowship, 1992; Fulbright Fellowship, 1994) working on biochemical aspects of ripening-related softening in tomato (Carrington *et al.*, 1993).

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APPENDIX

(Papers published in Refereed Journals)

ELSEVIER

Scientia Horticulturae, 57 (1994) 7–15

SCIENTIA HORTICULTURÆ

Growth, maturation and ripening of soursop (*Annona muricata* L.) fruit

Desmond B. Worrell[a], C.M. Sean Carrington*[,a], Donald J. Huber[b]

[a]*Biology Department, University of the West Indies, P.O. Box 64, Bridgetown, Barbados*
[b]*Horticultural Sciences Department, P.O. Box 110690, University of Florida, Gainesville, FL 32611, USA*

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Abstract

Flowers of soursop (*Annona muricata* L.) appeared to be protogynous and, following pollination, they entered a quiescent period of 6–15 weeks, during which time the stigmatic surface typically developed a sooty deposit. Further development was signalled by clumping of the carpels as the underlying tissue expanded. The time taken to reach fruit maturity from this 'take-off' point was found to be 15–21 weeks. Fruit growth was double sigmoidal. Maturity could be reliably detected when the density of the spurs on the fruit surface reached a minimum value (6 per 12 cm^2) and a slight paling of the initially dark-green skin occurred. This lightening of the skin probably reflected declining chlorophyll concentrations which fell to about 15% of their initial value. Mature fruit produced a biphasic respiratory climacteric, with CO_2 production reaching 100 ml kg^{-1} h^{-1} and then 350 ml kg^{-1} h^{-1} at 25–30°C. Peak ethylene production (250–350 ml kg^{-1} h^{-1}) occurred between the two respiratory maxima. The respiratory climacteric of harvested immature fruit tended to be higher and later than that of mature fruit.

Key words: *Annona muricata*; Fruit growth; Guanabana; Maturity; Ripening; Soursop

1. Introduction

The soursop, *Annona muricata* L., a native of Tropical America, is common throughout the islands of the Caribbean. There are over 100 species of *Annona* and several of these bear edible fruit (Mahdeem, 1990); however, only the cherimoya (*Annona cherimola*), custard apple (*Annona reticulata*), sugar apple (*Annona squamosa*) and soursop are of major commercial importance. Of these the soursop has the largest fruit and is the least cold-hardy (Morton, 1966). This

*Corresponding author.

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fruit, like that of other *Annona* species is a syncarp, and the white, juicy pulp has a delightful aroma and a somewhat sour-sweet, yogurt-like taste. The fruit is potentially useful as a processed commodity (Anonymous, 1975) but also has value as an exotic fresh fruit in distant markets, despite its extreme perishability (Seaton, 1988).

Soursop has a high postharvest respiration rate (Biale and Barcus, 1970; Lakshminarayana et al., 1974; Paull et al., 1983; Bruinsma and Paull, 1984; Lam and Zaipun, 1986) but information about the development of this fruit is limited. Like other *Annona* species, soursop is dichogamous but both protogyny (Venkataratnam, 1959) and protandry (Juliano, 1935; Kennard and Winters, 1960; Coronel, 1990) have been reported. An understanding of the process of fruit development and the identification of suitable maturity indices are important prerequisites for rational development and exploitation of this fruit crop. This study was undertaken to (a) characterise fruit growth patterns and (b) investigate possible maturity indices.

2. Materials and methods

2.1. Plant material

Fruit development was studied in a backyard orchard at Sedgepond, St. Andrew, Barbados, from October 1989 to May 1990 on ten soursop (*Annona muricata* L.) trees of a common, but unnamed cultivar. The trees were of unknown age and raised from seed. Fruit for respiratory studies were harvested in triplicate from the same trees at an immature (dark green with spur density of 10 spurs per 12 cm^2) or mature (turning pale green with spur density of 6 spurs per 12 cm^2) stage, weighed, washed and allowed to air-dry before use.

2.2. Fruit growth and development

About 300 flowers, at a stage where the outer petals had just begun to gape, were tagged, labelled and monitored over a 3 month period. Weekly measurement of fruit length and maximum diameter, as well monitoring of the timing of sepal abscission, fruit curvature, shoulder initiation and spur development, were carried out on blossoms that were tagged at the 'take-off' point (the stage at which growth commenced). Dry and fresh weights were measured from fortnightly harvests of six fruit, the dry weight being determined on half fruit dried in an oven at 100–120°C for 3 days. For chlorophyll measurements, six replicate samples, each of ten skin discs, were prepared from frozen fruit of known age using a 7 mm diameter cork borer. Discs were ground in a mortar and pestle using 80% (v/v) aqueous acetone, centrifuged, and assayed for chlorophyll in the supernatant by absorbance at 647 and 664 nm (Porra et al., 1989).

2.3. Postharvest changes

Individual fruit were placed in 25 l plastic bell-jars at room temperature (25–30°C) and ventilated with humidified air (approximately 1.5 l min^{-1}), the exit flow entering a 225-MK3 infra-red gas analyser (ADC, Hoddesdon, UK) via a

WA-161 multi-channel switching unit (ADC). Linkage to a microcomputer allowed automatic half-hourly data logging. Ethylene production was monitored twice daily for the same fruit by temporarily reducing the flow rate to 0.18 l min^{-1} and injecting a 1 ml air sample from the exit air stream into a Photovac gas chromatograph (Photovac, Ont., Canada). Both instruments were calibrated with appropriate certified standards (Matheson Gas Products, NJ, USA). Fruit were assessed visually for skin blackening and fungal growth and by finger pressure for softening. The percentage of the surface area showing such features was estimated using a clear vinyl overlay sheet with a 1 cm × 1 cm dot grid and noting the percentage of dots overlying such affected areas.

3. Results

3.1. Initiation of fruit development

Several developmental stages were identified in the transition from receptive flower to young fruit (Fig. 1). At the time that flowers had petals just beginning to gape (Fig. 1(A)), their stigmatic surfaces were wet but anthers had not begun to dehisce. Two to three days later, when the petals were fully open, or in some cases had abscised, the stigmatic surfaces were dry and pollen had been shed. Following anther dehiscence, the stamens abscised (Fig. 1(B)) and the flowers appeared to enter a resting phase characterised by a darkening of the stigmatic dome of the carpels (Fig. 1(C)). This state persisted in the majority of flowers for 6–15 weeks (Fig. 2(A)) and it was at this stage that about 70% of the tagged flowers abscised. The first noticeable sign of further activity was a slight swelling at the base of the flower, often accompanied by partial to complete loss of the apical black coloration. This resumption of growth, which we designated 'take-off' (Fig. 1(D)), was characterised by a cracking at the distal end of the blossom

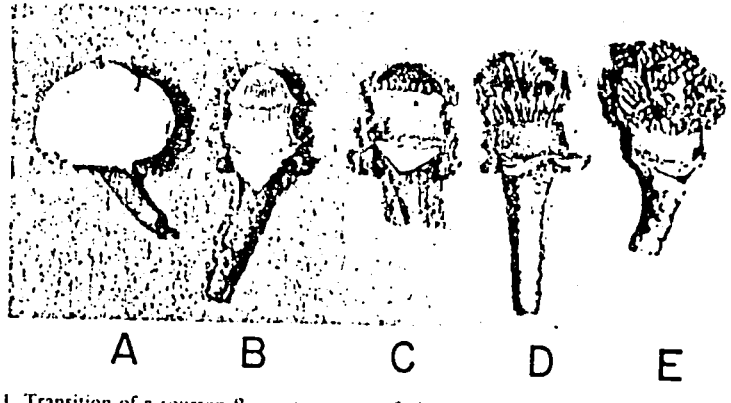

Fig. 1. Transition of a soursop flower to a young fruit (X1): (A) anthesis (petals have been removed); (B) anthers shed; (C) quiescent stage; (D) take-off point; (E) young fruit.

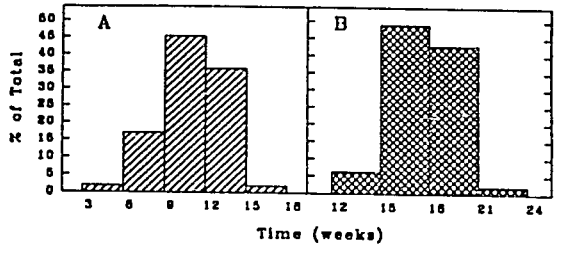

Fig. 2. (A) Time taken to reach take-off from anthesis in a sample of 53 soursop flowers. (B) Time taken to reach maturity from take-off in a sample of 49 soursop flowers.

with clumping of the carpels as the underlying tissues began to swell. Increases in length and diameter of the developing fruit then became evident (Fig. 1(E)).

3.2. Fruit growth

Length and diameter of soursop fruit were measured until 19 weeks after take-off, by which time some fruit had started to soften on the tree. Most fruit required 15–21 weeks from take-off to reach maturity (Fig. 2(B)). Whether size or weight was monitored (Figs. 3(A) and 3(B)), soursop displayed double-sigmoidal growth kinetics. Fruit reached half their final size by the end of the initial rapid growth phase which was followed by an intermediate 'resting' phase of about 4 weeks. The final growth phase, leading to full size and maturity, then occurred (Fig. 3(A)). When dry and fresh weights were monitored, the transition period between the two phases of rapid growth appeared to be a phase of reduced growth rather than no growth (Fig. 3(B)).

Soursop is a compound fruit formed as an aggregate of berries, and the individual constituent carpels persist as spurs on the fruit surface throughout development. The density of these spurs decreased from an initial value of 23 per unit sampling area (12 cm^2) to a final value of 6 per unit area in fully grown fruit (Fig. 3(C)).

3.3. Colour changes

Chlorophyll *a* and *b* concentrations in the fruit skin declined during the last 7 weeks of development (Fig. 4). This confirmed our observations that skin colour lightened as fruit matured, which we quantified as an initial value of 3/6 on Munsell Color Chart 5GY and as 6/6 at maturity. This lightening of skin colour began as pale striations radiating around each spur, which later spread and coalesced in fully grown fruit.

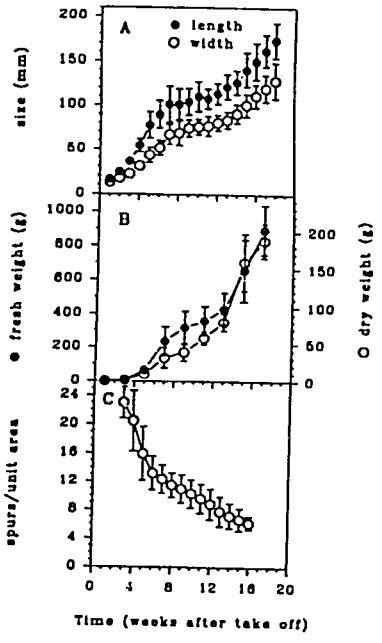

Fig. 3. (A) Growth of soursop fruit from take-off, as determined by length and width (mean ± SD of 15 fruit). (B) Growth of soursop fruit as determined by fresh and dry weight (mean ± SD of 15 fruit). (C) Growth of soursop fruit as determined by the number of fleshy spurs on the fruit surface per 12 cm^2 sampling area (mean ± SD of 15 fruit).

3.4. Morphological aspects of development

A number of qualitative aspects of fruit development were monitored. The three sepals of the soursop flower persisted to varying degrees during fruit development. While about 50% of the fruit monitored retained all sepals throughout growth, 7% had none at the start of growth and the remainder showed considerable variation in the timing and degree of sepal drop. Soursop fruit are characteristically curved, but the timing of the initiation of this curvature varied. Curvature tended to develop early, with about 33% of the fruit starting out curved and 44% developing curvature within the first 10 weeks after take-off. Soursop fruit also develop shoulders around the point of pedicel attachment. In most cases this began within the first half of growth. None of these parameters changed late enough or consistently enough in development to be of use as maturity indices.

3.5. Characterisation of the climacteric

Postharvest respiratory profiles were determined for triplicate fruit of two different stages of maturity (Fig. 5). The respiratory climacteric in the mature fruit

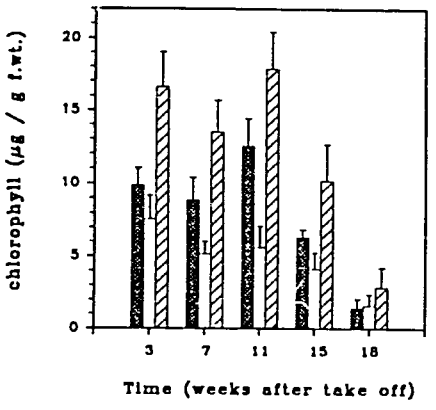

Fig. 4. Chlorophyll concentrations in the peel of soursop fruit during development (mean ± SD of six replicates): cross-hatching, Chl. *a*; blank, Chl. *b*; diagonal lines, Chl. *a* + *b*.

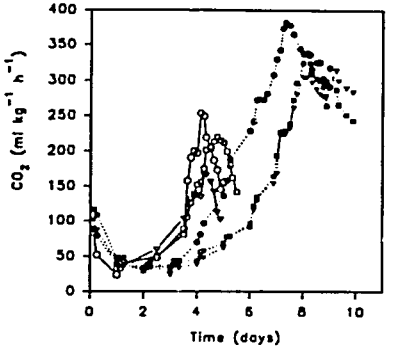

Fig. 5. Respiratory profiles of three mature (6 spurs per unit area) soursop fruit (open symbols) and three immature (10 spurs per unit area) soursop fruit (filled symbols).

reached a maximum of 150–250 ml CO_2 kg^{-1} h^{-1}, while the younger fruit gave respiratory peaks of 300–350 ml kg^{-1} h^{-1}. The climacteric occurred a few days later in the immature fruit than in the mature fruit.

Figure 6 shows the postharvest ripening behaviour of four mature soursop fruit. The respiratory drift of mature fruit exhibited a biphasic trend with the initial rise in CO_2 production reaching about 100 ml kg^{-1} h^{-1} followed by a brief lag

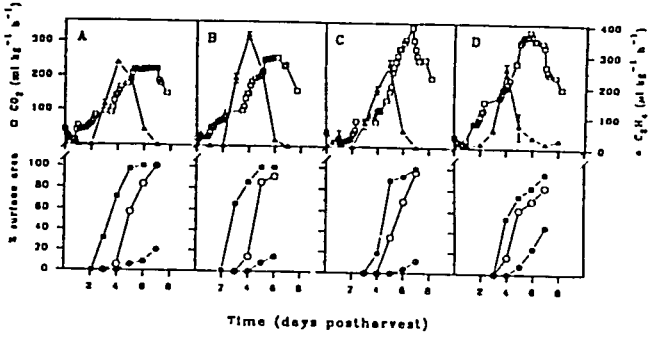

Fig. 6. Characterisation of postharvest ripening in four soursop fruit ((A)–(D)) as monitored by CO_2 and C_2H_4 production and by the percentage of the fruit surface showing softening, skin blackening and fungal growth: □, CO_2; ▲, C_2H_4; ■, softening; ○, skin blackening; ●, fungal growth.

before again increasing to a maximum of 250–350 ml kg^{-1} h^{-1} (Fig. 6). Ethylene production generally did not increase until after the first respiratory rise and reached a maximum of 250–350 ml kg^{-1} h^{-1}, between the two respiratory peaks. Softening seemed to begin with the C_2H_4 climacteric, all regions of the fruit fully soft by the time of peak C_2H_4 production. Skin blackening was coincident with the second phase of the respiratory climacteric. Toward the later stages of ripening, fungal growth became evident on the fruit surface.

4. Discussion

Our observations of an initially wet stigmatic surface prior to anther dehiscence, followed by a dry stigmatic surface at the time of pollen release 2 days later, suggest that soursop is protogynous, as reported by Venkataratnam (1959), and not protandrous as suggested by other workers (Juliano, 1935; Kennard and Winters, 1960; Coronel, 1990). The prolonged quiescent state of the pollinated flowers has not been reported previously, and does not occur in cherimoyas (M.L. Arpaia, personal communication, 1992). This resting period does not seem to be a result of correlative inhibition, where the development of newly pollinated flowers is suppressed by pre-existing developing fruit, since this quiescent phase occurs on trees without flowers or developing fruit. Furthermore, this lag occurs in flowers borne in both the wet and dry seasons, suggesting it may not be environmentally imposed. This phenomenon requires further study as it has important implications from a horticultural standpoint.

Double sigmoidal, triphasic growth such as that seen in soursop (Figs. 3(A) and 3(B)), comprising initial and final phases of rapid growth interspersed by a period of reduced growth, is relatively common in fruits and has been reported for blueberry, fig, grape, kiwifruit, persimmon, pineapple and all stone fruit

(Coombe, 1976; Monselise, 1986). Since pineapple and fig, like soursop, are compound fruits, but others in this list are not, double sigmoidal growth is not dependent on fruit type. In blueberry, the intervening lag phase is associated with embryo and endosperm growth, while in peach it is dominated by endocarp lignification (Monselise, 1986). It would be interesting to learn what internal changes characterise this phase in soursop.

Variation in final fruit size from 0.2 to 1.5 kg, probably related to the proportion of carpels fertilised, renders size unreliable for estimating maturity. Days from anthesis are also meaningless owing to the extended and variable post-anthesis quiescent period we have described. Our recognition of a take-off point from which fruit development should be monitored partly clarifies this variation; however, the time from take-off to full size and maturity varied by as much as 6 weeks (Fig. 2(B)). Neither sepal drop, fruit curvature nor shoulder development were useful indicators of maturity. In contrast, spur density was a reliable, size-independent measure of maturity, with 6 spurs per 12 cm^2, indicating full size attainment. We have successfully used this in conjunction with a slight paling of skin colour as maturity indices for this fruit.

Our finding of higher and delayed peaks of C_2H_4 and CO_2 production in immature fruit compared with mature fruit (Fig. 5) differs from that of Lam and Zaipun (1986), who found no consistent differences in the postharvest C_2H_4 and CO_2 production in the two maturation stages. This discrepancy may reflect a greater difference in maturity (10 vs. 16 weeks after take-off) in our fruit relative to those used in the other study (10 vs. 12 weeks). The biphasic respiratory climacteric reported for soursop by earlier workers is confirmed although our CO_2 values were invariably twice those previously reported (Biale and Barcus, 1970; Lakshminarayana et al., 1974; Paull et al., 1983; Bruinsma and Paull, 1984). Similarly, our values for C_2H_4 production were higher than those of previous reports (Paull et al., 1983; Bruinsma and Paull, 1984; Lam and Zaipun, 1986). In both instances, our higher measuring temperatures might account for these differences, or even our use of a flow-through rather than a static system for gas analysis. A further difference relates to the kinetics of C_2H_4 evolution. Paull et al. (1983) and Bruinsma and Paull (1984) showed the C_2H_4 peak coincident with the final, major respiratory peak while our fruit showed peak C_2H_4 production between the two respiratory peaks (Fig. 6), as shown for cherimoya (Kosiyachinda and Young, 1975; Brown et al., 1988). The surface microbial growth reported (Fig. 6) is problematic and was no doubt facilitated by the high humidity within the flow-through system. We believe, however, that this was limited and occurred too late to compromise the C_2H_4 or CO_2 data. The eventual decrease in CO_2 production and the postclimacteric return of C_2H_4 production to preclimacteric values support this interpretation.

Soursop is eaten soft but at a stage where there is no skin blackening or, at most, when this is only beginning. Since this period of prime consumption quality coincides with the end of the first respiratory climacteric, respiration monitoring may be of some value in predicting the timing of optimum eating quality. Future

efforts must address extending the preclimacteric postharvest life of this highly perishable fruit.

Acknowledgement

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ARTICLES, ABSTRACTS AND NEWS

The Waiting Game of Soursop

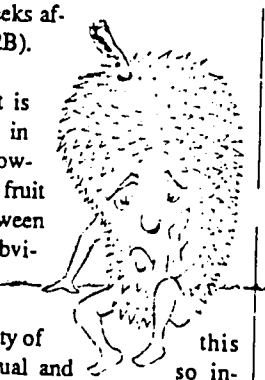
by Desmond B. Worrell and C. M. Carrington, Biology Department, UWI, P.O. Box 64, Bridgetown, Barbados

LE JEU DE PATIENCE DU COROSSOL

Le corossol, *Annona muricata*, très commun dans la Caraïbe, a une biologie florale très particulière; après la pollinisation et la chute des anthères la surface stigmatique de la masse carpellaire se recouvre d'un dépot noiratre. la fleur entre alors dans un état de quiescence qui peut durer de 9 à 15 semaines avant que le fruit ne commence à se développer. Le fruit atteint alors la maturité au bout de 15 à 21 semaines. Différentes hypothèses sont envisagées.

Soursop, *Annona muricata* L, is a common backyard tree throughout the Caribbean. It is native to Tropical America and was probably spread through the region by pre-colombian inhabitants of these islands. The fruit is an aggregate of berries, the shape and furrowing of the fruit determined by the uniformity of the natural pollination process.

Our attempts to quantify fruit development in soursop were initially frustrated by the very variable rates at which individual flowers proceeded through to fruit development. The situation became much clearer once careful observations were made of flowers from anthesis onwards. Several stages were identified in the transition from receptive flower to young fruit (Fig. 1). Flowers with petals gaping were used as the starting point or anthesis stage (Fig. 1A). The mass of anthers forming a rim below and around the central mound of carpels then abscise (Fig. 1B) and soon the stigmatic surfaces of the carpels develop a black sooty deposit (Fig. 1C). Flowers can remain in this state of apparent quiescence for weeks to months. The commencement of fruit growth is heralded by the splitting of the blackened stigmatic surface into clumps as the underlying tissue begins to grow, a stage we have termed 'take-off' (Fig. 1D). Once this stage has been reached growth proceeds in a double sigmoidal fashion from what is now recognizable as a young fruit (Fig 1E). It seems that most flowers spend some 9-15 weeks in the apparent quiescent state depicted in Fig. 1C before entering the take-off phase (Fig. 2A) and the fruit finally reach full size 15-21 weeks after take-off (Fig. 2B).



It is unclear what is actually going on in these quiescent flowers. While in most fruit there is a lag between pollination and obvious fruit development it is the extent and variability of this lag which is unusual and so intriguing. It does not seem to be simply a case of correlative inhibition where freshly-pollinated flowers must wait their turn on the 'runway' until older flowers 'take-off' or mature fruit abscise. Flowers appearing on trees without developing fruit or flowers also go through the same 'inactive' phase. Nor is the lag dependent on whether flowers are borne in the wet season or the dry season. Lastly, flowers of soursop have long been known to exhibit dichogamy (Juliano 1935) but stigma receptivity and anther dehiscencence are out of synchrony by about a day and it is hard to see how this can relate to the long lag phase we have described. This phenomenon deserves further study and we hope the identification of these distinct, early phases of soursop fruit development will bring more predictability to the management of this promising fruit crop.

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Reference

Juliano, J. B. 1935. Morphological contribution on the genus Annona Linnaeus. Philippine Agriculturist 24:528-541.

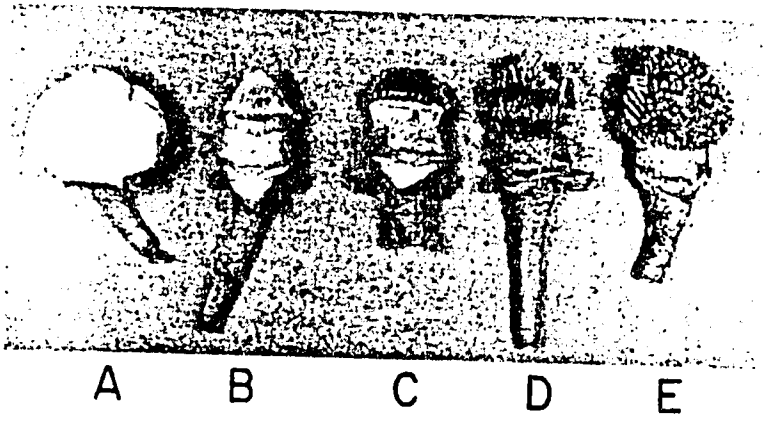

Fig. 1. Receptive flower to young fruit.

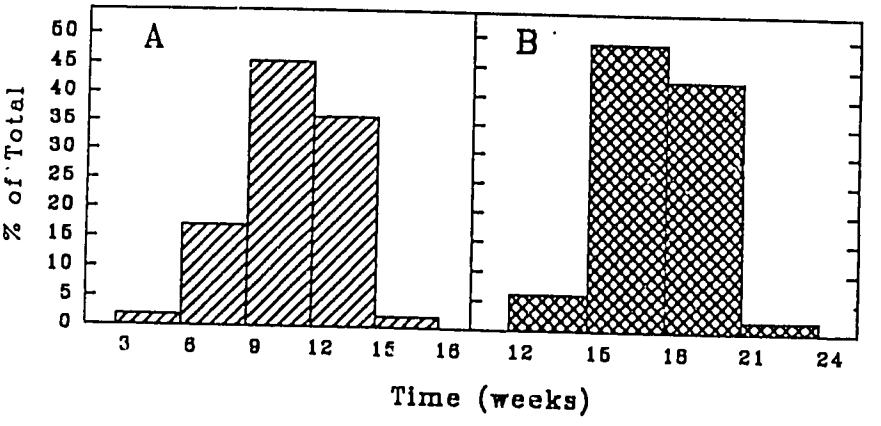

Fig. 2A. Time taken to reach 'take off' from anthesis in a sample of 53 soursop flowers.
Fig. 2B. Time taken to reach maturity from 'take off' in a sample of 49 soursop fruit.