STORAGE AND RIPENING OF PLANTAINS

by

Joseph Bongay George

A thesis submitted for the degree of Doctor of Philosophy of the University of London and for the Diploma of Imperial College.

Department of Botany and Plant Technology,
Imperial College,
London SW7.

Tropical Products Institute,
56/62 Gray's Inn Road,
London WC1.

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ABSTRACT

Physiological factors likely to affect the storage and ripening of plantains (Musa AAB) at ambient temperature of 20°C have been investigated, using Ghanaian plantains of a French-type cultivar, Apem. The pre climacteric phase as measured by following either rates of CO_2 evolution in single plantain fingers or colour change of the peel was used as an indicator of the potential storage life.

Of all the factors tested, water stress had the largest effect on the preclimacteric period of plantains. Typically, an increase in weight loss of 0.5% per day was accompanied by a decrease of 65% in the potential preclimacteric period of cultivar Apem and of two other plantain cultivars. Under similar conditions of water stress, plantains were more sensitive than bananas. The preclimacteric period was extended when the storage temperature was reduced, but 14°C was the lowest temperature where fruits were free of chilling injury, which was observed in green fruits at 10° and 6°C. Treatment with gibberellin extended the preclimacteric period when subsequent storage was under conditions favouring low weight loss (i.e. at high humidity), but had no effect under water stress (i.e. at low humidity). Treatment with aqueous $CaCl_2$ extended the preclimacteric period, but salicylhydroxamic acid had no effect.

An examination of the co-ordination of ripening changes showed that water stress and gibberellin had no effect on the respiratory patterns, but water stress increased the maximum rate of ethylene production and also the ACC content of the pulp. The synchronisation of ripening of pulp and of peel remained unchanged when the preclimacteric period was reduced by water stress and when it was extended by treatment with gibberellin ($GA_{4/7}$). However, there was a transient delay in peel

colouration and pulp softening in stressed fruit.

The mode of action of water stress in the preclimacteric period was investigated by measuring ethylene production and respiratory rates of unripe fruits and respiratory rates and preclimacteric period of fruits treated with low concentrations of ethylene. To measure production of stress ethylene by unripe fruits, other methods such as ACC and accumulation in a closed system were tested and discarded in favour of ethylene trapping in a continuous flow system using mercuric perchlorate. Water stress markedly increased both ethylene production and respiratory rates of preclimacteric plantains. Treatment with ethylene reduced the preclimacteric period and increased respiratory rates of preclimacteric plantains. The effects of exogenous ethylene on the preclimacteric period and respiratory rates were quantatively similar to the effects of water stress on the preclimacteric period and on respiratory rates. Thus, the large effect of water stress on the preclimacteric period of plantains is probably mediated wholly or largely through increased synthesis of endogenous ethylene in response to stress.

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CHAPTER 1

INTRODUCTION

1.1. General Introduction

Banana belongs to the family of Musaceae which consists of two main genera - Ensate and Musa. The genus Musa is composed of four sections. Nearly all edible bananas are derived from two wild diploid species - Musa acuminata (AA) and Musa balbiasiana (BB) (Simmonds, 1966). Diploid, triploid and tetraploid groups of M. acuminata are derived from the wild AA group and most edible cultivars are triploid. No diploid (BB) form of M. balbisiana is known which has edible or parthenocarpic fruits and no triploid of M. balbisiana (BBB) exists.

Plantain (Musa AAB group) and cooking banana (Musa ABB) are also triploid and they are derived by AA x BB hybridisation. Plantain comprises of two main forms: French plantain with seven to ten hands per bunch and numerous fingers and Horn plantain with three to five hands per bunch and fewer individual large fingers (Burden and Coursey, 1977). 'Apem' (Plate 1) is the local name of the French type of plantain which was mostly used in the present studies and its characteristics have been fully described by Karikari (1970, 1973). Apantu and Osa, both of the Horn type, were other cultivars used in experiments described in this thesis (Plate 1).

Plantains and cooking bananas (Musa AAB and ABB) are an







Photographs showing three cultivars of plantains

(a) Apem (French type)

(b) Apantu (Horn type)

(c) Osa (Horn type)

important staple food in many regions of the humid tropics and small quantities are exported to Europe, especially Britain where it is consumed by non-European immigrant groups.

The Food and Agriculture Organisation (FAO) estimated the world production ofplantains in 1976 at 18 million tonnes per annum which could have increased since that date. In Ghana where the material for this study was grown about 1 million tonnes per annum are produced (Karikari, 1970).

The utilisation of plantains is highly influenced by traditional cooking practices: unripe (green) fruits are usually boiled or mashed, occasionally roasted; ripe fruits are boiled or sliced and fried.

The total volume of plantains imported into Europe and United States is difficult to assess as total trade statistics from known supplying countries are often inaccurate. In Britain alone, imports are currently estimated at between 2,500 and 10,000 tonnes per annum mainly re-exported from Holland (unpublished data).

Fruits for both domestic and export markets are harvested firm and green, but ripening can easily commence during collecting and transportation, thus the storage life is usually short and serious losses often occur. Despite these losses, very little research into the post-harvest physiology

and biochemistry during storage and ripening of plantains has been undertaken, though a lot is known about the behaviour of dessert bananas of the Musa AAA group.

The subject of post-harvest physiology and biochemistry of bananas including plantains has been reviewed in the past (Loesecke, 1949; Simmonds, 1966; Palmer, 1971) and more recently a comprehensive review has appeared (Marriott, 1980); consequently this review is limited to areas relevant to the present studies. These include:

1. the preclimacteric period and the role of exogenously and endogenously produced ethylene (stress), temperature and growth regulators in its control.
2. the mechanism of co-ordination of the climacteric.

The greater part of the review will be based on selected publications on bananas, on which more work has been done, but attempts will be made to review all published data on the relevant topics on plantains.

1.2. Measurement of the preclimacteric period

The preclimacteric period of fruits including bananas and plantains is defined as the time which elapses between harvest and the initiation of ripening, (ie to the start of the climacteric). This period has been shown to be a useful physiological measurement of the storage life of bananas (Peacock and Blake, 1970). In the preclimacteric phase, bananas and plantains are usually green and very firm. In this state the fruits can be successfully stored as they are

less susceptible to both mechanical damage and fungal infection. When the climacteric commences, the fruits rapidly lose both their green colour and firmness becoming more susceptible to both mechanical damage and fungal infection, and thus have a very short shelf life. In general, therefore, the length of the preclimacteric phase is a good indicator of the storage potential of bananas and plantains. In this thesis the term "preclimacteric period" will be used to mean the storage period or green life.

Previously, the effect of various storage conditions on the storage life of bananas was assessed by measurement of the proportions of ripe hands within replicated groups after a fixed period, selected according to the desired transit time, typical periods of 15, 20 and 25 days have been used (Simmonds, 1966). This method lacks a quantitative basis of comparison of storage life under different conditions. In recent investigations, the preclimacteric periods of single fruit fingers have been measured and data from these investigations show that the preclimacteric period is closely coordinated within a single bunch. For a bunch, the preclimacteric period increases from the proximal to the distal end of the bunch, thus the sixth hand has a preclimacteric period 30% longer than the first hand. At the Sandy Trout Food Preservation Laboratory, investigations involving the effect of various storage conditions including the effect of temperature, water stress, ethylene and other factors on bananas have been measured by observing the preclimacteric period of single fingers ventilated at a constant rate with humidified ethylene-free air (eg Blake

and Peacock, 1971; Littmann, 1972a,b,c; Peacock, 1973a, b; Peacock and Blake, 1970).

Karikari et al (1979) have also examined the duration of the preclimacteric period of individual fingers of plantains (cv. Apem) using fingers both from different hands within a bunch and from different bunches of different ages. They observed significant differences between hands of the same bunch and between bunches of different ages but observed no difference in both the pattern of climacteric respiration and mean maximal rates of CO_2 production between hands of the same bunch or between bunches of different ages.

This approach requires a high degree of replication, but provides estimates of the separate effect of each stress while avoiding effects caused by interaction between different fingers. It can also be used to measure the interaction between fruit maturity at harvest and different storage treatments, since significant differences could be observed within bunches by dividing and randomising fingers from adjacent hands between treatments.

1.3. Effect of temperature on the preclimacteric period

Investigations on the effect of temperature (Peacock and Blake, 1970) showed that, over a small range of temperature close to the optimum for storage (12.5-21.5°C) the preclimacteric period in bananas was an inverse linear function of temperature, with the temperature coefficient in the range of 1 to 3 days for 1°C. This coefficient is generally un-

affected by fruit maturity. For a given temperature change, the proportional change in the preclimacteric period is constant irrespective of the maturity at harvest. A similar observation has been made on bananas of dessert clones (Musa AAAA group) stored at 12 and 14°C (Marriott et al, 1979). On the contrary, Blake and Peacock (1971) observed that at a broader temperature range (15-35°C) the relationship between the duration of the preclimacteric phase and temperature is logarithmic: $\log g = MT + C$, Where g is the preclimcteric period in days at temperature T and M and C are constants. This relationship which implies a constant proportional reduction in the preclimacteric period for a given increase in temperature is in agreement with the observations by Peacock and Blake (1970) that proportional changes are independent of fruit maturity, though for a particularly immature bunch (cv. Giant Cavendish) it was observed that the preclimacteric period was greater at 17° than at 15° or 13°C. It is likely that at lower temperatures (13° or 15°C) chilling injury occurs in the fruits and the less mature bunches are more susceptible than more mature ones. If this hypothesis is valid it may explain why Marriott and New (1975) also observed a reduction of 1.1 day in the preclimacteric period of bunches of "Valery" stored at 12°C when compared with those stored at 14°C. It should be further noted that these temperatures with the exception of 17°C are within the range (10 - 15°C) at which chilling injury is known to occur. Lizana (1975) observed that at 40 - 42°C ripening was normal (ie the climacteric occurred), though the preclimacteric period was the same at 35°C, but at 50°C ripening was not normal. This is in agreement with Blake and Peacock (1971) who observed that ripening may be partially suppressed at high temperatures.

There have been two limited studies on the effect of temperature on plantain storage (Hernandez, 1973; Guillemot, 1976). Hernandez (1973) has shown that plantains stored at 12°C had their preclimacteric periods extended when compared with fruits stored at ambient temperature (25°C) and when similar fruits were stored in sealed polythene bags containing ethylene absorbent at 12°C the preclimacteric period was further extended to 55 days. Similarly, at 12°C the preclimacteric period of plantains which was previously 12 days was extended to 65 days when fruits were stored in sealed polythene bags containing ethylene absorbent (Guillemot, 1976). Green plantains were damaged by chilling at 8°C (Hernandez, 1973) and at 10°C (Guillemot, 1976), but remained undamaged at 12°C in either investigation. Bananas and plantains may have a similar temperature range for ripening and and may have a Similar temperature coefficient.

1.4. Effect of ethylene on preclimacteric period

1.4.1. Effect of exogenously applied ethylene

The effect of exogenous ethylene on the preclimacteric period of bananas and plantains is critical to the understanding of the onset of the climacteric and the technology of bulk storage. Trace quantities of ethylene shorten the preclimacteric period and high concentrations rapidly induce the climacteric. In Table 1 is a summary of some of the published data on the effect of low concentrations of ethylene on the preclimacteric period

of bananas. Ethylene concentrations as low as 0.01 ppm can reduce the duration of the preclimacteric period and this reduction is usually a logarithmic function of ethylene concentration. Liu (1976a) observed a consistent response of bananas to 0.1 ppm ethylene and an intermediate but significant response to 0.015 and 0.05 ppm, which are lower than the internal concentration of 0.06-0.20 ppm in the fruit. Peacock (1972) observed that the sensitivity of bananas stored at 20°C to ethylene decreased as the fruit maturity increased, when ethylene treatment was applied immediately after harvest. He, however, further observed that when fruits were treated at different periods (1-6 days) after harvest, the sensitivity to ethylene increased with increasing time between harvest and the commencement of ethylene treatment. Both the data of Liu (1976a) and Peacock (1972) are not in agreement with the hypothesis of Burg and Burg (1965) that there is a threshold concentration of ethylene approximately equal to the internal concentration below which exogenously applied ethylene is ineffective and further testing of this hypothesis is required. Liu (1976b) treated bananas with a high concentration of ethylene (10 ppm) for short periods (up to 28 hours) soon after harvesting. He observed that there was a minimum length of time (threshold length of time) below which the preclimacteric period was unaffected and above which the climacteric was induced at once. For 29 bunches harvested over a wide range of maturity, he found a good correlation between the preclimacteric period and the threshold length of time which could be used as a rapid test to predict the pre-

Table I

The effect of low concentrations of ethylene on the storage of bananas.

a) Cv. Valery, bunch age 111 days, at unspecified temperature were continuously exposed to ethylene as stated and colour scored to 2.

b) Cv. Giant Cavendish, at 21°C fruits were continuously exposed to ethylene as stated and respiration rate measured.

c) Cv. DwarfCavendish, at 20°C fruits were exposed to 0.44 ppm ethylene for 4 days and green life determined - not determined.

Ethylene treatment (ppm)	Source		
	Palmer (1971)[a]	Liu (1976)[b]	Peacock (1972)[c]
	Preclimacteric Period (days)		
0.00	16	13.5	38.9
0.01	-	9.5	-
0.05	-	6.5	-
0.10	10	3	-
0.50	3.5	1	26.4
1.0	2	1	-

climacteric period. These data also suggest that conditions exist when bananas can be exposed to high concentrations (10 ppm) of ethylene for a limited period (up to 24 hours) without any effect on the preclimacteric period. In contrast, Peacock (1972) observed that exposure of bananas to 0.4 ppm ethylene for a period of 1 - 6 days caused a reduction in the preclimacteric period proportional to the period of exposure, but exposure to 60 ppm ethylene for 6 hours had no effect. Both Peacock (1972) and Liu (1976b) proposed that for a limited period after harvest banana fruits remain relatively insensitive to ethylene as has been reported for avocados (Gazit and Blumenfield, 1970). This is consistent with the hypothesis by Burg and Burg (1965) that when a fruit is attached to a tree, it receives a ripening inhibitor which suppresses its response to ethylene.

Recently New (1976) and Marriott et al (1979) have similarly observed that very mature fruits harvested prior to ripening in the field may still be stored 10 to 20 days prior to the onset of the climacteric. Further work into factors which determine this insensitivity of freshly harvested bananas to ethylene is advocated as it may help to explain changes leading to the onset of the climacteric.

Although the ripening of plantains can be induced by ethylene treatment in a manner comparable to that used for dessert bananas, the sensitivity of plantains to ethylene has only been tested at high concentrations (ie 1,000 ppm) (Sanchez-Nieva et al, 1970; Karikari et al, 1979). It is, however, not known whether at low concentrations plantains will respond to ethylene in a manner similar to bananas.

1.4.2. Effect of stress ethylene

Many tissues including those of preclimacteric plantains and bananas which usually evolve little or no ethylene show a surge in ethylene production 3 to 10 times the basal level upon applying treatments such as wounding, extremely high temperatures and, exposure to light which impose physiological stress on the tissues. Ethylene so produced is called stress ethylene or sometimes wound ethylene - if stress is caused by wounding. Littmann (1972a) carried out detailed investigations into the effect of water loss (stress) during banana storage in which other variables such as temperature and ventilation were controlled. Preclimacteric bananas were compared at two humidities - low (13.1 ± 4.0% relative humidity) and high (95.0 ± 1.8% relative humidity). He observed a linear relationship between the rate of weight loss and the preclimacteric period, with a reduction in the preclimacteric periods of about 30% at a maximum weight loss of 1.2% per day. In other investigations he also observed that the rate of ethylene production of bananas under stress increased by factors between two - three compared with unstressed fruits (Littmann, 1972b). Similarly, he observed that during stress the respiratory rate during the preclimacteric period was increased (Littmann, 1976c) and the climacteric peak depressed. He proposed that ethylene production, as a result of physiological stress, was the mediator of both respiratory rise and the decrease in preclimacteric period. However, ethylene may not be the sole mediator as other growth hormones may be involved. If Litmann's conclusion is valid, then it would be consistent with the sensitivity of bananas to prolonged exposure to low con-

centrations of ethylene already discussed (Peacock, 1972; Liu, 1976 a).

Thompson et al (1974) also investigated the effect of water stress on the ripening of plantains. In a series of experiments in which preclimacteric fruits were stored under low and high relative humidity, they observed that at low humidity with a higher rate of water loss per day the rate of ripening was greatly increased.

Maxie et al (1968) carried out investigation on the effect of irradiation on the ripening of banana fruits. Fruits from two cultivars - Gros Michel and 'Valery' were given various doses of gamma irradiation and respiration rates, ethylene production and other ripening parameters determined in both unirradiated and irradiated fruits. They also investigated the effect of injury on the ripening and the interaction between physiological stresses such as mechanical injury and irradiation. Both ripening and the onset of ethylene production was delayed by 15 to 23 days when fruits were treated with 35 krad dose. Similarly, irradiation (50 krad) delayed the onset of ripening in injured fruits by several days but did not completely eliminate the wounding stimulus. When fruits were treated with 1,000 ppm ethylene for 24 hours at 62°F and subjected to 35 krad, they needed 12-24 hours longer treatment with 1,000 ppm ethylene than unirradiated fruits before they reached a comparable stage of ripeness. Maxie and co-workers suggested that the inhibition of ripening caused by irradiation involves a decrease in sensitivity to the ripening action of ethylene. They further suggested that the accelerated

ripening observed in fruits resulting from mechanical injury may have been caused by increased ethylene production, though they were unable to detect increased ethylene production. This may explain why Peacock (1973a) got a smaller reduction (10%) in the preclimacteric period with less severe injuries such as bruises and scratches.

1.5. Biosynthesis of ethylene and its mode of action

1.5.1. Biosynthesis of ethylene

Most investigations into the biosynthetic pathway for ethylene during fruit ripening have been largely restricted to temperate and sub-tropical fruits. Thus, little or no work has been done on ethylene biosynthesis in either bananas or plantains. Even where studies on ethylene biosynthesis have been undertaken in other fruits our understanding of the nature of the pathway or pathways is limited by our inability to purify the enzymes by the usual procedures. Past reviews (eg Abeles, 1973; Lieberman, 1975; Yang, 1974; Yang and Pratt, 1978; Lieberman, 1979) should be consulted for more detailed discussion. Biosynthesis of ethylene in most fruits is via the oxidation of L-methionine and Burg and Clagett (1976) have demonstrated the ability of ripe banana fruits to oxidise carbon 3 and 4 of L-methionine to ethylene. In apple tissues, methionine is metabolised via s-adenosyl methionine (SAM) as an intermediate in the pathway to ethylene (Adams and Yang, 1977). The evidence for SAM as an intermediate was obtained after feeding ^{14}C methionine into apple tissues and identifying 5-methyl thioadenosine (MTA) and methylthioribose (MTR)

which correlated with ethylene production. When ethylene production was inhibited by aminoethoxyvinyl-glycine (AVG), no MTA or MTR was detected in tissue extract. Preclimacteric apple tissues did not produce MTA or MTR from ^{14}C labelled methionine, this is understandable as preclimacteric tissue does not normally produce ethylene. These provided some evidence that SAM may be an immediate precursor in the conversion of L-Methionine to ethylene. Adams and Yang (1978) studied the metabolism of ^{14}C methionine in apple plugs held in nitrogen and in air. In nitrogen a radio-active compound designated X accumulated, which when fed to apple plugs in air produced the radio-active ethylene. The compound was identified as 1-aminocyclopropane-1-carboxylic acid (ACC). ACC has been extracted from bananas and other tissues and determined by conversion to ethylene (Hoffman and Yang, 1980). Previous studies also show that ACC stimulated ethylene evolution in different tissues, including fruit tissues from a wide range of plant species (Cameron et al, 1979). Although no tracer studies have been reported on bananas, it is probable that the major biosynthetic pathway for ethylene in this fruit is from methionine via s-adenosylmethionine and ACC. Earlier reports of biosynthesis of ethylene in ripe banana fruits from β-hydroxypropionate via acrylic acid (Shimokawa and Kasai, 1970a,b,c) may be an alternative but less important pathway as ACC is widely distributed in fruits.

Present evidence also shows that most stress ethylene is derived from L-methionine, perhaps by the same biochemical pathway similar to basal ethylene. However, during severe

wounding, ethylene may be produced by peroxidation of linolenic acid which also results in some ethylene production (Lieberman and Kunishi, 1968).

Recent studies (Mattoo and Lieberman, 1977) show that apple slices gradually lose the ability to synthesise ethylene when they are incubated with a mixture of enzymes that digest the cell wall. The released protoplast did not produce ethylene, but after culturing for several days, ethylene production was observed concomitant with the generation of the cell wall material. Protoplast will produce ethylene if supplied with methionine and the system was inhibited by AVG and N-propyl gallate. Lieberman (1979) quotes an unpublished data by Andersen in which protoplasts obtained by less drastic lytic methods produce ethylene from ^{14}C labelled methionine in complete absence of cell wall, thus suggesting that ethylene forming system is localised on the surface of the plasma membrane.

1.5.2. Mode of action of ethylene

Information on the mode of action of ethylene in bananas is indirect and fragmentary. Kidd and West (1945) suggested that ethylene was a fruit-ripening hormone and that a minimum threshold concentration is necessary to exert physiological activity in fruit tissues. The effects of various concentrations of exogenous ethylene on the preclimacteric period of whole bananas have been reviewed in 1.4.1. and this should be con-

sulted for a more detailed discussion. Vendrell and McGlasson (1971) observed that when banana slices were treated with ethylene, the onset of climacteric was accelerated and the ethylene production during the climacteric was also stimulated, and post-climacteric ethylene production was partially suppressed relative to the untreated slices. The inhibition of post-climacteric ethylene production was a function of ethylene concentration (5-10 ppm) and the duration of ethylene treatment (0.5-3 days). A similar inhibition of ethylene production in post-climacteric bananas, in which ripening was induced by propylene, has been observed by McMurchie et al (1972). Consequently, McMurchie and co-workers suggested that in bananas, two systems regulate ethylene biosynthesis. In naturally ripening fruits, ethylene biosynthesis is initiated by some factors in the regulation of ageing process - system 1. The ethylene produced by this system reaches a level in the fruit sufficient to initiate the respiratory climacteric and associated ripening changes. The rise in respiration is accompanied by a further increase in ethylene production - system 2. Although system 1 is circumvented if bananas are treated with either ethylene of propylene, system 2 is then partially suppressed. McMurchie et al (1972) suggested that the suppression of ethylene synthesis by system 2 could not be a simple end product inhibition, as it persisted even after the end of the ethylene treatment. Preclimacteric ethylene production in bananas (New, 1976) and in plantains (Karikari et al, 1979) is low and usually undetectable, but a sudden onset and increase in synthesis occur prior to the climacteric increase in respiration. The maximum ethylene evolution in plantains is much greater (about

10 fold) than for dessert bananas whether related to fresh weight or dry weight (Karikari et al, 1979).

Salkveit et al (1978) have shown that when Ag(1) as aqueous $AgNo_3$ is applied to bananas both ethylene synthesis and the response to exogenous ethylene were inhibited. As this inhibition was not reversed when fruits were treated with ethylene, they suggested that Ag(1) may have interferred with the primary action of ethylene in the banana tissue. A similar effect of Ag(1) on ethylene production in a different fruit tissue has been reported (Janes and Frenkel, 1978). Data of Salkveit and co-workers may give an insight into the complexity of ethylene action, but do not give a clue to the actual nature of the cellular binding site or sites in bananas. However, various studies on ethylene binding sites in other tissues have been undertaken and these have been reviewed elsewhere (Lieberman, 1979).

Both the peel and pulp of banana produce ethylene during ripening, but the latter produces more ethylene than the former (Vendrell and McGlasson, 1971). Ethylene production in the peel occurs only during senescence (ie climacteric). Unlike the pulp, the peel does not show any respiratory rise during the climacteric comparable to the whole fruit, although a reasonable transient respiratory rise can be induced with ethylene (Terai et al, 1974). In previous studies Terai et al (1973) also showed that in bananas treated with ethylene at one end, those girdled by removal of peel at the centre evolved markedly less ethylene at the untreated end than did ungirdled bananas. They suggested that the peel tissue plays an important part in ethylene diffusion. This diffusion can be important

in fruit ripening.

Although various reasons have been advanced for the role of stress ethylene in fruit ripening (Matsuno and Uritani, 1972; Vendrell, 1970b), the actual role is still not fully understood. Salkveit and Dilly (1978) suggested that the damped oscillation curve for wound ethylene production in pea stem tissue they observed may reflect a return to a proper hormonal balance and that stress ethylene may be the symptom of hormonal imbalance induced as a result of metabolic stress.

1.6. Effect of growth regulators

Since fruit ripening is a developmental process under hormonal control, emphasis is now being placed on the role of hormones other than ethylene. Vendrell (1969, 1970a) observed that auxin applied by vacuum infiltration delayed ripening in green banana slices and delayed but increased peaks in ethylene production and rate of respiration. In contrast, when the whole fruit was dipped in auxin, ripening was accelerated. Apparently, when auxin was applied as a dip very little penetrated the pulp, but when the slices were vacuum infiltrated both the peel and the pulp were penetrated. Vendrell (1970a) concluded that although auxin stimulated ethylene production in the slices, it extended the preclimacteric period by increasing their resistance to ethylene induced ripening. He confirmed this by treating both previously auxin infiltrated and uninfiltrated slices with 10 ppm ethylene for 24 hours. Auxin infiltrated slices needed an extra 24 hours treatment with ethylene before reaching comparable colour with untreated slices.

Similarly, gibberellins can extend the preclimacteric period of bananas. Leonce (1974) and Kapoor and Turner (1976) observed a considerable increase in the preclimacteric period of bananas when individual fingers were dipped in gibberellins. There was more response to GA 4/7 at 10 ppm than to GA 3 at 100 ppm. Vendrell (1970b) also studied the effect of GA 3 on the ripening of bananas. Whole fruits and slices were dipped and vacuum infiltrated respectively with GA 3 (10^{-5}M to 10^{-2}M). In one experiment slices were treated with ethylene (10 ppm) for 24 hours and later treated with gibberellic acid. Gibberllic acid did not affect ethylene production in either the slices or the whole fruit, but ripening was delayed in the whole fruit in contrast to the tissue slices in which it was accelerated. The aspect of ripening delayed in the whole fruit was the yellowing of the peel. A similar delay in the peel yellowing was observed when previously treated ethylene slices were treated with gibberellic acid. Thus, Vendrell (1970b) suggested that gibberellic acid may have opposite effects in the peel and pulp. In the peel gibberellic acid may induce or increase the production of a substance (or substances) which when translocated into pulp may cause a delay in the process of ripening. Further work into the nature of this substance or substances is needed.

1.7. Co-ordination of the climacteric

Studies on the integration and control of ripening in climacteric fruits including bananas and plantains have been centred around the role of ethylene. Ethylene is considered to trigger biochemical changes associated with ripening, though of late

other growth hormones such as auxins and gibberellins have been implicated. Previous studies were limited by the difficulty in following some of the biochemical changes in intact fruits. For instance, test solutions cannot penetrate very easily the tissue of green bananas and plantains. However, Palmer and McGlasson (1969) observed that normal ripening occurs in slices cut transversely across both peel and pulp, with a thickness of 6 mm. These slices could be vacuum infiltrated with test solution without causing major disturbance to their metabolism. When the slices are maintained under sterile conditions with or without ethylene they pass through a preclimacteric period and then a climacteric phase apparently comparable to the intact fruit.

Previous studies have, however, shown that slicing of fruits causes major injury and stress which will affect ripening (Laties, 1963; Ap. Rees, 1968; Mcglasson and Pratt, 1964). This probably led to the development of a technique for vacuum infiltration of labelled metabolites into intact fruits (Frenkel et al (1969). The effect of stress including wounding on ethylene production in fruits has been reviewed (1. 5. 2.).

1. 7. 1. Protein synthesis

Data on bananas and on other fruits show that RNA and total protein content do not change drastically during ripening (eg Richmond and Biale, 1967; Wade et al, 1972), although Palmer (1971) reported data of Sacher which showed a significant increase in total protein content about 5 days before onset of the climacteric. Various investigations into protein

synthesis during ripening in banana slices infiltrated with ^{14}C amino acids have been carried out (eg Brady et al, 1970a, 1976; Wade et al, 1972). From this study it was first demonstrated that during the early part of the climacteric following ethylene production, both the rate of uptake of amino acids into cells and the rate of incorporation in protein increased (Brady et al, 1970a). There was redirection of protein synthesis as shown from changes in soluble protein observed after gel electrothroesis and the inhibition of ripening by protein inhibitors. Similar changes in protein patterns after gel electrophoresis have been reported elsewhere (Skakoun and Daussant, 1973). However, Wade et al, (1972) observed no change in protein content relative to the total level of nitrogen in the pulp tissue. They further showed that there was no evidence for movement of nitrogen between the peel and the pulp tissues during ripening and concluded that no substantial increase in the amount of ribosomal materials occur in the early climacteric. These conclusions were based on the fact that they observed no change in both total content of RNA or the base content of RNA in pulp tissue. More recently, Brady and O'Connel (1976) treated banana slices with ethylene (5 ppm) for durations of 6 and 12 hours and compared the rates of protein synthesis. Treatment for 6 hours produced only a small and transient respiration response so that the climacteric occurred at the same time as in untreated slices, whereas when slices were treated for 12 hours the climacteric was rapidly induced. They found that changes in protein synthesis were very similar for the two periods of ethylene treatment and concluded that the redirection of protein synthesis they observed was a response to ethylene

treatment rather than to ripening. They also used double labelling experiments to establish that the increase in protein synthesis involves the redirection of large range of soluble protein and is not limited to the synthesis of a specific protein. They concluded that there is as yet no evidence that the synthesis of a particular protein not previously present is triggered by ethylene treatment which leads on to ripening. Conflicting results have been reported on the change of total pectin methylesterase (PME) activity in banana, especially in the cultivar Gros Michel. Hultin and Levine (1965) reported a four-fold increase, with a water soluble fraction (1), but Deswardt and Maxie (1967) found no change in total PME activity. Brady (1976) extracted, purified and characterised this enzyme. It had a molecular weight of about 30,000. After isoelectric focussing, the enzyme was found in two peaks at pH 8.8-8.9 and 9.3-9.4. The enzyme in the two peaks had the same molecular weight and showed only minor difference in response to pH, salt concentrations, etc. during assay. He suggested that the multiple forms previously reported (Hultin et al, 1966) may have developed during extraction and fractionation and the two fractions he obtained could have arisen similarly. Further work is needed to resolve this conflicting report. An increase in peroxidase activity during the climacteric has been reported (Haard, 1973). This increase in peroxidase activity was associated with the particulate fractions which increase in activity three-fold during the climacteric rather than the soluble fraction in which activity did not change appreciably. The isoenzymes of peroxidases have been partially characterised from ripe bananas (Nagle and Haard, 1975). It is,

however, not clear whether changes in peroxidase activity reported reflect the synthesis of new protein.

1.7.2. Permeability and lipid changes

The theory that fruit ripening results from marked increase in membrane permeability with accompanying cellular decompartmentation of enzymes and substrates is still an influential idea in studies on fruit ripening. Previous permeability studies on banana have been reviewed elsewhere (Palmer, 1971). Brady et al (1970b) have shown that in banana slices treated with ethylene the respiratory response commenced within 8 hours after ethylene treatment but changes in tissue permeability were not measurable until after 32 hours. This suggests that tissue permeability may not have caused the respiratory rise. Wade and Bishop (1978) have also shown that the content of total lipid in banana fruit pulp tissue remained constant during ripening induced by ethylene, but the fatty acid composition of the lipid changed. Physical measurement showed that motion and fluidity of lipid layers also increased during ripening. Recently, Wade et al (1980) studied tissue permeability and membrane lipid composition in whole banana fruit treated with propylene. They found a temporary correlation between the change in membrane lipid composition and the onset of rapid increase in leakage of ions from the tissue. Though leakage of ions increased progressively 44 hours after propylene treatment, there was no further increase in the

proportions of linolenic acid between 36 and 68 hours. Wade and co-workers concluded that the increase in the leakage of ions observed in the pulp tissue of banana was caused by either changes in the membrane lipid composition or changes in other factors, such as tissue water potential. The involvement of the latter, however, remain unknown. Rao and Bandyopadyay (1978) have also studied changes in the component of fatty acids in pulp lipids of two cultivars of bananas and in one cultivar of plantains, of the French type. Total lipid composition in bananas and in plantains were almost identical and did not change during ripening. But there were increases in fatty acid composition especially in palmitic and linoleic acids as ripening progressed. This is in agreement with other reports that during ripening there are changes in fatty acid composition in banana (Brady et al, 1970b; Wade and Bishop, 1970) and Awan and Ndubizu (1978) indicate that changes in plantains are similar.

1.7.3. Respiratory changes

During ripening bananas and plantains exhibit a climacteric pattern of respiration (Loesecke, 1949; Biale, 1960; Palmer and McGlasson, 1969; Karikari et al, 1979). After harvest at 20°C, the respiratory rate within 2 to 3 days rises from a steady value of about 20 mg/Co_2/kg/h in the green hard banana fruit to about 125 mg/Co_2/kg/h at the climacteric peak and then falls to about 100mg/Co_2/kg/h as ripening proceeds. Considerable variation from this typical pattern has been reported. Some fruits pass through the classic typical preclimacteric minimum (Tager and Biale, 1957); in others the respiratory rate rises con-

tinuously from harvest until the start of the climacteric (Peacock and Blake, 1970) and in some fruits, during the preclimacteric period there is a downward drift (New, 1976). On the average the increase from preclimacteric to climacteric peak varies from four to ten-fold depending on the cultivars and storage conditions.

In plantains of the French type stored at 25°C, the preclimacteric rate of CO_2 evolution fell from 44 mg/kg/h to 24 with an average rate of 0.74 mg/kg/h per day. In both plantains and bananas the increase in climacteric respiration is usually preceded by increased synthesis of ethylene and the composition of gases in the storage atmosphere affect both ethylene and CO_2 evolution (Young et al, 1962; Mapson and Robinson, 1966; Quazi and Freebairn, 1970). Mapson and Robinson (1966) carried out studies into the effect of low oxygen tension on both ethylene and CO_2 evolution and other ripening changes in bananas. They observed that at 18°C the rise in climacteric respiration and ethylene production did not occur in bananas unless the oxygen concentration of the storage atmosphere was above 7.5 - 8.0%. At concentrations between 5 - 7.5%, the onset of ripening was delayed, but when 2.7 ppm ethylene was included into the storage atmoshphere the rise in respiratory climacteric and other ripening changes occurred. They concluded that the delay in ripening of banana fruits held at low oxygen tension was due to the inability of the fruit to synthesise ethylene and was not caused by the inhibition of the associated respiratory changes. If this conclusion is valid then the stage in ethylene synthesis likely to be inhibited is the conversion of 1-aminocyclopropane-1-carboxylic acid

(ACC) to ethylene) as present evidence suggests that the conversion of ACC into ethylene is more critically dependent upon oxygen (Adams and Yang, 1979) than other stages in the pathway for ethylene synthesis (see 1.5.1.). McGlasson and Wills (1972) have investigated other causes of the inhibition of ripening in bananas stored at low oxygen tension (controlled atmosphere). They held green banana fruits in humidified gas streams comprising air as control, A (5% CO_2, 20% O_2, 75% N_2); B (0% CO_2, 3% O_2, 97% N_2); and C (5% CO_2, 3% O_2, 92% N_2). Compared with air, A, B and C delayed ripening and the greatest delay was in C with the lowest oxygen concentration. Although A, B and C reduced the rates of oxygen uptake, but because of delay in the preclimacteric period, the total oxygen uptake was increased during the preclimacteric period. Although increases in organic acid content occurring within the first four days of applying treatment to the fruits were variable in A, B and C, the largest change in acids was within the first day of the treatment. By application of the cross-over theorem of Chance to these data, they suggested that low oxygen limited the operation of Krebs cycle between pyruvate and citrate, and 2-oxoglutarate and succinate. No control points to explain the inhibition caused by carbon dioxide were apparent. The oxygen levels reported by both Mapson and Wills (1966) and McGlasson and Wills (1962) exceed the level of oxygen necessary to saturate (0.01 - 0.2%) the cytochrome oxidase. Further investigation into O_2 penetration into the fruit tissue may resolve the discrepancy between the oxygen requirement for respiration in the whole fruit and that for cytochrome oxidase. McGlasson et al (1971a, b) studied metabolic pathways in banana slices using infiltration of both ^{14}C labelled metabolic

intermediates and of metabolic inhibitors. Both approaches indicated that the tricarboxylic acid (TCA) cycle and the pentose phosphate pathway are the main pathways of respiration following slicing of preclimacteric bananas and also during the early stages of ripening. Although both monofluoroacetate and cycloheximide inhibited ripening, the respiratory climacteric still occurred. In contrast, inhibitors such as potassium cyanide (CN) which inhibited respiratory climacteric invariably inhibited other ripening parameters. Thus McGlasson and co-workers (1971b) concluded that although the respiratory rise is not itself dependent on other ripening changes, it is a pre-requisite for the initiation of the non-respiratory components of ripening. This is not in agreement with previous reports that the respiratory climacteric in some fruits including bananas can be stimulated without affecting the non-respiratory components of ripening (Vendrell, 1969; Janes and Frenkel, 1968; Frenkel et al, 1968). Other workers (eg McGlasson and Wills, 1972; Saliminen and Young, 1975; Terai and Ogata, 1976) have also advanced evidence for the utilisation of the TCA (Cycle) during the climacteric. In a series of investigations, Solomos and Laties (1974, 1976) observed that potassium cyanide as well as ethylene stimulated the climacteric and ripening in a wide range of fruits. They observed that ethylene responsive fruits (climacteric fruits) were invariably CN resistant. Recently, Theologis and Laties (1978) have tested this hypothesis in apples and bananas. They investigated the effect of specific respiratory inhibitors using banana slices from three stages of the climacteric. In the intact fruit CN induced respiration and caused ripening as in natural or

ethylene ripened fruit. CN and antimycin, selective inhibitors of the cytochrome chain, increased the respiratory rate of preclimacteric slices, but m-chlorobenzydroxamic acid (CLAM) alone did not. CLAM and CN together inhibited respiration, thus confirming the operation of the cyanide resistant oxidase in the respiratory process. In the presence of weak acid uncouplers of oxidative phosphorylation, the respiratory rates of preclimacteric slices were increased by a factor which was 15 times that of the intact climacteric fruit, suggesting that a huge unexpressed respiratory capacity exists in the intact preclimacteric fruit. Mid-climacteric slices were also cyanide-resistant and the respiratory rate was sharply inhibited by CLAM in the presence of CN. Titration data of coupled and uncoupled slices (from the preclimacteric fruit and mid-climacteric periods) with CLAM in the presence and absence of CN suggested that during both the preclimacteric and mid-climacteric phases, respiration was mediated by the cytochrome oxidase pathway. They concluded that the capacity of this pathway is sufficient to sustain the climacteric and there is no evidence for the involvement of the cyanide resistant oxidase.

During ripening there is an increase in the respiratory rates of both the peel and the pulp. Data of Terai and Ogata (1977) showed that the respiratory activities of both the peel and the pulp are enhanced by ethylene treatment, but unlike the pulp respiratory activity of the peel is not sustained thereafter. This response to ethylene does not involve the synthesis of new respiratory enzymes and may be mediated by the enhancement of the TCA pathway comparable to the situation in the pulp.

Despite the effort that has gone into trying to understand the

role of respiration in fruit ripening in general and in banana ripening in particular, we still do not understand fully the role respiration plays in ripening.

1.7.4. Starch-sugar conversion

The hydrolysis of starch and accumulation of sugars are the most striking compositional changes which occur in bananas and plantains during ripening (Loesecke, 1949). Starch which forms about 85-95% of the dry matter of green banana pulp is degradated rapidly at the onset of ripening, such that the ripe pulp contains 5-15% starch and 70-80% sugars. The sugars are mostly glucose, fructose and sucrose. Data from other studies (Kawabata and Sawayama, 1974; Roma and Patwardhan, 1976) are consistent with this pattern. Enzymes involved in carbohydrate metabolism have been studied as well (Kumar and Sanwal, 1977; Shukla et al, 1973; Sigh and Sanwal, 1975; Desai and Deshpande, 1975; Young et al, 1980). Young et al (1980) determined the rate of conversion of starch to sugar as well as the activity of the enzymes responsible for starch hydrolysis at various stages of the climacteric of banana fruit. Starch hydrolysis did not commence until the respiration rate had increased to two-thirds of the climacteric rate and this coincided with the peak of ethylene production. Activities of both amylases and phosphofructokinase started to increase when the respiratory rate of the fruit had increased to more than one-half of the climacteric level. The increase in activities of these enzymes did not involve de novo synthesis of the enzyme proteins. Since starch hydrolysis occurred well after the climacteric, Young and co-workers suggested that starch hydrolysis

could not have initiated the climacteric. They concluded that during ripening phosphofructokinase is activated and that its affinity for substrate changes, with control (through a negative co-operative effect) becoming less stringent as ripening progresses. The change in enzyme activity is sufficient to account for the increase in respiratory activity during the climacteric.

During ripening, changes in starch and total free sugars in plantains are consistent with the pattern in bananas (Stratton and Loesecke, 1930; Marriott et al, 1981), but starch hydrolysis is slower and less complete than in bananas (Simmonds, 1966; Marriott et al, 1981). The starch content of fully ripe plantains has been variously reported as low as zero/fresh weight and as high as 28.6%/fresh weight (Ketiku, 1973). More recently, the starch content in fully ripe plantains has been reported as 9%/fresh weight (Marriott et al, 1981). Further investigation may resolve these discrepancies reported. At a similar stage of ripeness plantains are firmer and have more dry matter than bananas (Marriott et al, 1981).

1.7.5. Acid changes

The pH of banana pulp falls during ripening from about 5.4 (± 0.04) in the green fruit to about 4.5 (± 0.31) in the ripe pulp (Palmer, 1971). The titratable acid (8 meq/100 g fresh tissue) of plantains is about twice that of dessert bananas (Loesecke, 1949). Previous data reviewed by Palmer (1971) and recent ones (Kawabata and Sawayama, 1974; Shimokawa et al, 1972) are in agreement that malic, citric, oxalic are the main organic acids in bananas. Both Shimokawa et al, 1972 and Urado and Ogata (1976) have studied changes in oxalic acid content in banana tissue homogenates during ripen-

ing. The activity of the enzyme which was an oxalate oxidase was higher in climacteric than in preclimacteric fruit.

1.7.6. Pigment changes

During ripening chlorophyll content of banana peel decreases from between 50 and 100 µg/g/fresh weight to almost zero and no appreciable changes in carotenoids fractions occur (Loesecke, 1949). Similar degradation of chlorophyll has been observed in bananas in which ripening was initiated by ethephon (Srinivasan et al, 1973). Gross et al (1976) have also studied changes in carotenoid composition of the peel and the pulp of ripe bananas. The peel-carotenoid concentration was 5-6 µg/g/fresh weight and consisted of mainly α-carotene (7%), β-carotene (14%) and lutein (56%). The concentration of carotene in the pulp was between 0.6-1 µg/g/fresh weight and consisted of α-carotene (31%), β-carotene (28%) and lutein (33%). Studies on the activity of the enzyme, chlorophyllase show that the activity of this enzyme closely follows a pattern identical to the climacteric respiration (Looney and Patterson, 1967). In plantains studies on changes in pigment during ripening have been confined to pulp carotene. Paul and Southgate (1969) gave values of carotene content as 60 and 200 µg/g for ripe pulp of plantains and bananas respectively. The value for plantains is unexpectedly low, as although some ABB group of cooking bananas do not have a white flesh, most true plantains (AAB group) have an orange-tinted pulp. Moreover, data from earlier studies (Asanjo and Porrata, 1956) show carotene content of 1040 and 670 µg/mg in green and ripe fruits respectively.

1.8. Conclusion

In general data in this review shows that the post-harvest physiology and biochemistry of plantains show many similarities to dessert bananas of the AAA group, in chlorophyll degradation, tissue softening, in at least partial hydrolysis of starch to sugar (Sanchez-Nieva et al, 1970; Marriott et al, 1981), in ripening in response to high concentration of ethylene (Sauchez-Nieva et al, 1970; Karikari et al, 1979) and in the extension of the preclimacteric period in response to decreasing temperature or storage in low oxygen tension (Guillemot, 1978; Hernandez, 1973). During ripening, however, plantains differ from dessert bananas in ethylene production (Karikari et al, 1979); in firmness, starch-sugar conversion and in dry matter (Marriott et al, 1981). However, unlike bananas, only limited detailed work has been done on plantains to justify such similarities and differences. It is with this intention that the present studies directed at investigations into the effect of various treatments including humidity and growth regulators on the storage and ripening of plantains at ambient temperature was undertaken.

CHAPTER 2

MATERIALS AND METHODS

2. 1. Materials

Plantain fruits, mostly cultivar 'Apem', were produced at the University of Ghana, Agricultural Research Station, Kade, using standard practices (Karikari, 1971). Dates of fruit emergence and harvesting for each bunch were recorded. Bunches were cut and packed as individual fingers and labelled according to hand and bunch and air freighted to Tropical Products Institute (TPI), UK. At Kade, fingers were treated with benomyl (1,000 ppm) and selected fingers were also treated in TPI at 35°C prior to commencement of storage. Bunches not immediately used for experiments were stored at 14°C and relative humidity of 80-90%. Except where specified, plantains were of the cultivar Apem and bananas obtained from commercial sources were typical fruits of the Cavendish sub-group, probably cultivar Robusta.

2. 2. Methods

2. 2. 1. Biochemical methods

2. 2. 1. 1. Chlorophyll determination

Chlorophyll content of the peel was determined by the method of Holden (1976). Five discs were cut from the middle of a finger and were immediately put into a sample bottle containing

10 ml acetone/water mixture (80:20, v/v). Duplicate samples were taken from each fruit. The discs were then homogenised in the acetone/water solvent, using successive volumes of the solvent until no traces of green colour were left in the residue. The extract was filtered through a Buchner funnel and the final volume made up to 15 ml. The filtrate was centrifuged at low speed for 10 minutes in a centrifuge (series MSE, Super Minor). The amount of chlorophyll was determined spectrophotometrically (spectrophotometer series SP 6-400 Pye Unicam) at 663 nm and 645 nm wavelengths. These wavelengths corresponded to absorption maxima for chlorophyll a and b respectively. Since the spectra for a and b overlap on these wavelengths, chlorophyll content was calculated using equations derived by Mackinney (1941).

$$(\text{Chlorophyll a}) = 0.0127 + A_{663} - 0.00269 + A_{645}$$

$$(\text{Chlorophyll b}) = 0.0227 + A_{645} - 0.00468 + A_{663}$$

Where A = absorbances and the resulting concentrations are in g/1.

2. 2. 1. 2. Total soluble solids

Total soluble solids were determined by the method described by Wade and Bishop (1978) in which 2 g of fresh pulp tissue was homogenised in 10 ml distilled water, taking duplicate samples from each fruit. The homogenate was centrifuged at low speed for 20 minutes using the centrifuge described in 2. 2. 1. 1. 2-3 drops of the supernatent were used for determining total soluble solids using a refractometer (Series Abbe 60 A76191, Bellingham and Stanley Ltd., Tunbridge Wells, UK).

2. 2. 1. 3. Assay for 1-amincocyclopropane-1-carboxylic acid (ACC)

ACC was extracted by the method described by Boller et al (1979): 2 g of freeze-dried tissue (peel or pulp) was extracted with 20 ml of cold 80% ethanol containing 0. 05% (v/v) mercapto-ethanol. The sample was thoroughly homogenised and the homogenate centrifuged for 25 minutes at 12,000 x g in a centrifuge (series MSE high speed 18). The supernatent was concentrated in vacuo at 38°C to dryness and made up to the final volume of 1 ml. Serial dilutions of standard ACC were also made. The extract and standard ACC solutions were assayed using the method of Lizada and Yang (1979). To 0. 1 ml of the extract or standard ACC solution in a 25 ml test tube, 0. 1 ml 10^{-6}M $HgCl_2$ was added. The volume was brought up to 0. 9 ml with distilled water and the test tube was sealed. A duplicate sample was taken from each extract or ACC standard. The test tubes were kept at 0°C. Approximately 0. 1 ml of a cold mixture of 5% Na OCl and saturated Na OH (2. 1, v/v) was injected through the stopper using a 1 ml syringe fitted with a 25 gauge needle. The reaction was vigorously agitated for about 1 minute. After 2. 5 minutes it was again agitated before 5 ml of gas sample was withdrawn. Ethylene was then analysed as described in 2. 2. 2. 3. Internal standards were used to determine the conversion of ACC to ethylene in the extract. An extract was spiked with a known concentration of ACC and conversion of ACC to ethylene in the tissue calculated. Conversion of ACC to ethylene in the tissue was about 55%.

2. 2. 1. 4. Ethylene trapping

Ethylene was trapped in mercuric perchlorate using the method of Ward et al (1978).

Respired gas streams from each finger were bubbled at a constant rate (4l/h) through a fine sintered glass head in a Quick fit tube (V. 100 ml). The tube contained 20 ml of 0. 25 M mercuric perchlorate solution in 2. 0 M perchloric acid. The mercuric perchlorate solution was prepared by dissolving 10. 8 g red mercuric oxide in 40 ml perchloric acid containing 10 ml of water in a flask kept at 0°C. After dissolving all the mercuric oxide the solution was made to a final volume of 200 ml by adding water. No filtering was required.

After trapping ethylene for a period of 24 hours , each collection tube was removed and the sintered glass head rinsed with a few drops of the solution. Additional solution was added to make a final volume of 25 ml. Each tube was made air-tight to preclude further absorbtion of ethylene from the air. Equivalent samples were collected as blanks using ethylene-free air.

For liberating ethylene from the ethylene/mercuric perchlorate complex, 4 ml of the aliquot was pipetted into a 250 ml flask taking duplicate samples at each time and the flask was flushed with nitrogen gas for about 5 minutes. The nitrogen gas displaced ethane from the flask and thus minimised contamination. The flask was made air-tight using a suba seal. 6 ml of 2.0 MHCl was gently injected into the flask through the seal. After 15 minutes, a gas sample (about 5 ml) was withdrawn through the seal using an air-tight glass syringe and ethylene analysed as described in 2. 2. 2. 3. In preliminary investiga-

tions, the efficiency of trapping was determined by trapping various known concentrations of ethylene varying from 0.01 to 1 ppm. After trapping, the ethylene was released and efficiency of trapping calculated. The mean trapping efficiency was 75-80% (see results).

2.2.2. Gas handling and analysis

2.2.2.1. General gas handling system

Figure 1 is the generalised schematic diagram of the handling system. Two pumps drew fresh air from the fresh air inlets. Pressure governors reduced the air pressure to a suitable level and a throttle situated between the two lines equalised the pressure. Two further throttles, one in each line, controlled the flow rates. All these flow rates and pressure control throttles were pre-set.

The air in each channel was then passed through two successive tower filters with 'pellets' of purafil to scrub ethylene. Ethylene-free air at equal pressure and flow rates emerging from the outlet was humidified as described in 2.2.2.6. and then passed to two sets of manifolds, one in each storage cabinet. From each manifold, the air was passed onto a set of 12 sealed containers, each with a single finger. The respired gas streams were then passed to a gas handling system (the Analytical Development Company, ADC, Hoddesdon, Herts, UK), which fed each stream of air to the carbon dioxide analyser. From the CO_2 analyser the sample gas was re-routed through the gas chromatograph via a pneumatically operated switch

valve. During an automatic cycle, the dwell time for each channel was 7.5 minutes in a three hour cycle. In studies in which a flow through system was not used, fruits were put on shelves of the storage cabinets. The cabinets were periodically ventilated by opening the doors for a brief period (approximately 5 minutes) every day.

2. 2. 2. 2. Carbon dioxide analysis

CO_2 was analysed using an infrared gas analyser (series 200,310, ADC, Hoddesdon, Herts, UK) with two ranges 0-02% and 0-1%. The analyser had devices for both manual and automatic switching from one range to the other. The analyser was calibrated thus:- the zero setting was on CO_2 free air, ie air which had been stripped of CO_2 was routed to the analyser cell. 0.16% CO_2 in air from a cylinder incorporated into the system was used for setting the sensitivity of the analyser. CO_2 readings from each fruit were continuously recorded using a chart recorder or a digital print-out (see Figure 1 for a description of the gas handling system).

2. 2. 2. 3. Ethylene analysis

For ethylene analysis, the respired gas was fed through a gas sampling valve (V. 1 ml), and a sample was automatically injected into a gas chromatograph fitted with a flame ionisation detector (Series 1400, Varian Aerograph, Walton-on-Thames, Surrey, UK) and with either a porapak type or an alumina column. (series F1-8200). The column and the detector temperatures

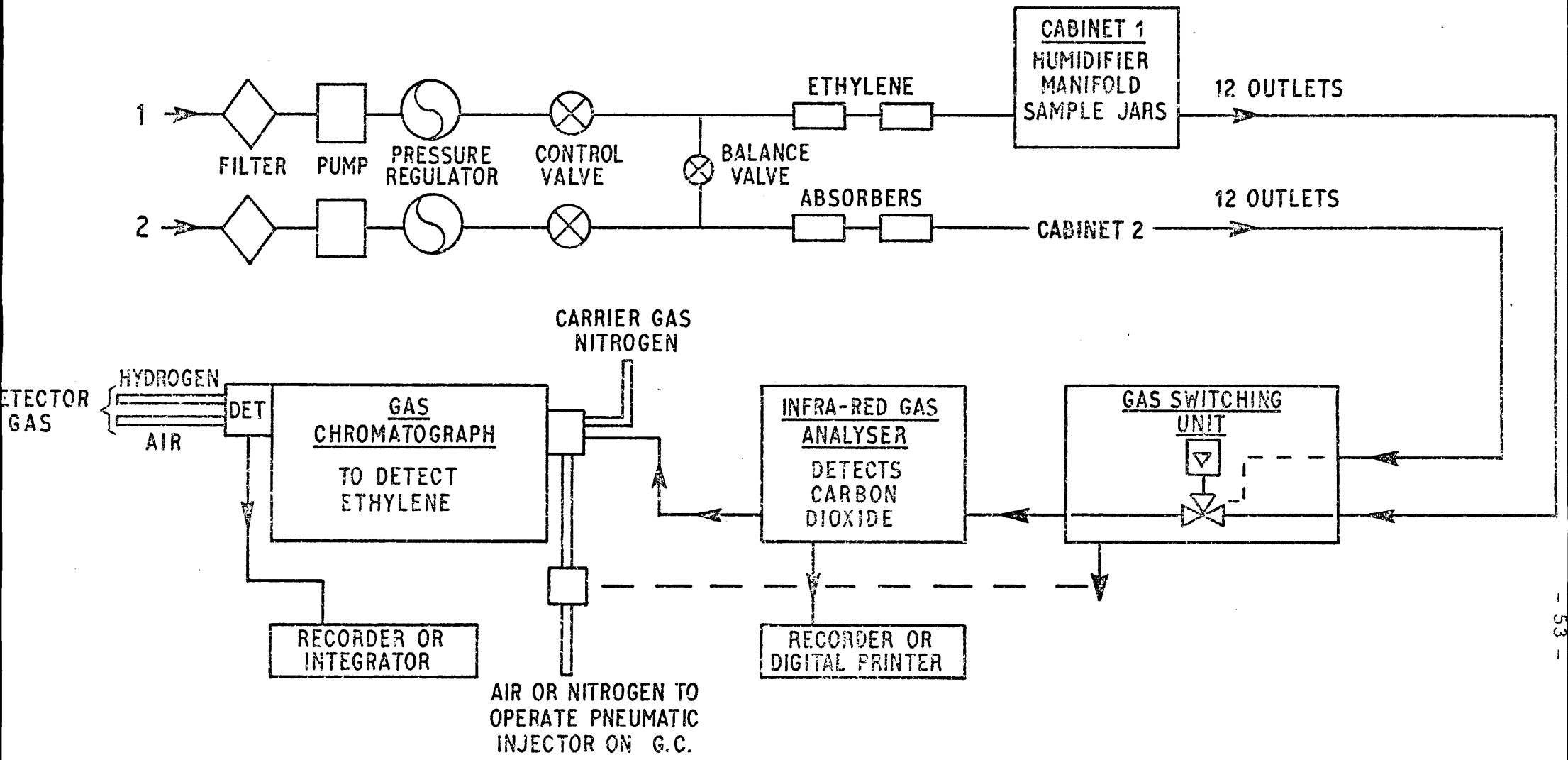


Figure 1: GENERALISED SCHEMATIC DIAGRAM OF A CONTINUOUS FLOW THROUGH SYSTEM

were 80-90°C and 250°C respectively. The injection temperature was kept low as ethylene being a gas did not require vapourisation. Flow rates of gas streams (ml/min) were: nitrogen (carrier gas) 15; hydrogen 20; and air 350. Ethylene coming out of the gas chromatograph was measured as peak height using a chart recorder or an integrator (Hewlett Packard, 3380A). For most of the studies the gas chromatograph was fitted with a porapak column and ethylene peak heights were measured using a chart recorder.

In later studies involving static sampling, an alumina column was used and peak heights of ethylene were measured using an integrator. The alumina column gave a better separation of ethylene and ethane and thus minimised the interference by ethane (Ward et al, 1978).

2. 2. 2. 4. Determination of ethylene in preclimacteric fruits

This simple method for extracting ethylene from within the tissue was a modification of the sampling procedure adopted by Musgrave et al (1972). Each finger was submerged beneath an inverted funnel, the stem of which was capped with a suba seal. While the fruit was still beneath the funnel it was cut into two halves and each half was squeezed using a hand screw developed for bananas. Gas bubbles liberated from the fruit accumulated in the stem of the funnel. A sample of the gas (3 ml) was withdrawn through the top using an air-tight syringe and ethylene was analysed as described in 2. 2. 2. 3. At least three fingers were sampled from each treatment.

2. 2. 2. 5. Ethylene dilution system

Ethylene dilution system in Figure 2 was a slight modification of the one described by Saltveit (1978). The resevoir was filled with 5% ethylene in nitrogen from a cylinder by displacing air from the jar. The lid of the jar was then replaced and clamped. All joints were first made air-tight and the transparent tubing joining the two ends of the tygon tubing (0. 8 cm diameter) also greased to make it less porous to ethylene. Lengths of the tygon tubing were varied according to the ethylene concentration required. The carrier gas was ethylene free air bubbled through water to almost full saturation. Excess moisture in the carrier gas was first trapped in a jar before it was passed through the dilution system. Ethylene diffusing through the tygon tubing was diluted by altering the flow rate of the carrier gas. The concentration of ethylene in the effluent gas was directly proportional to the length of the tygon tubing and inversely proportional to the carrier gas flow rate. As the lengths of the tubes were fixed, ethylene concentration was controlled by altering the flow rate of the carrier gas which were continuously monitored on meters. In the Table 2 are typical combinations of flow rates of the carrier gas and lengths of the tygon tubing corresponding to the ethylene dilution required.

2. 2. 2. 6. Control and analysis of water vapour

For most of the studies low and high humidities only were used. The effect of vapour pressure deficit (VPD) was determined by measuring the rate of weight loss of each fruit. Each finger

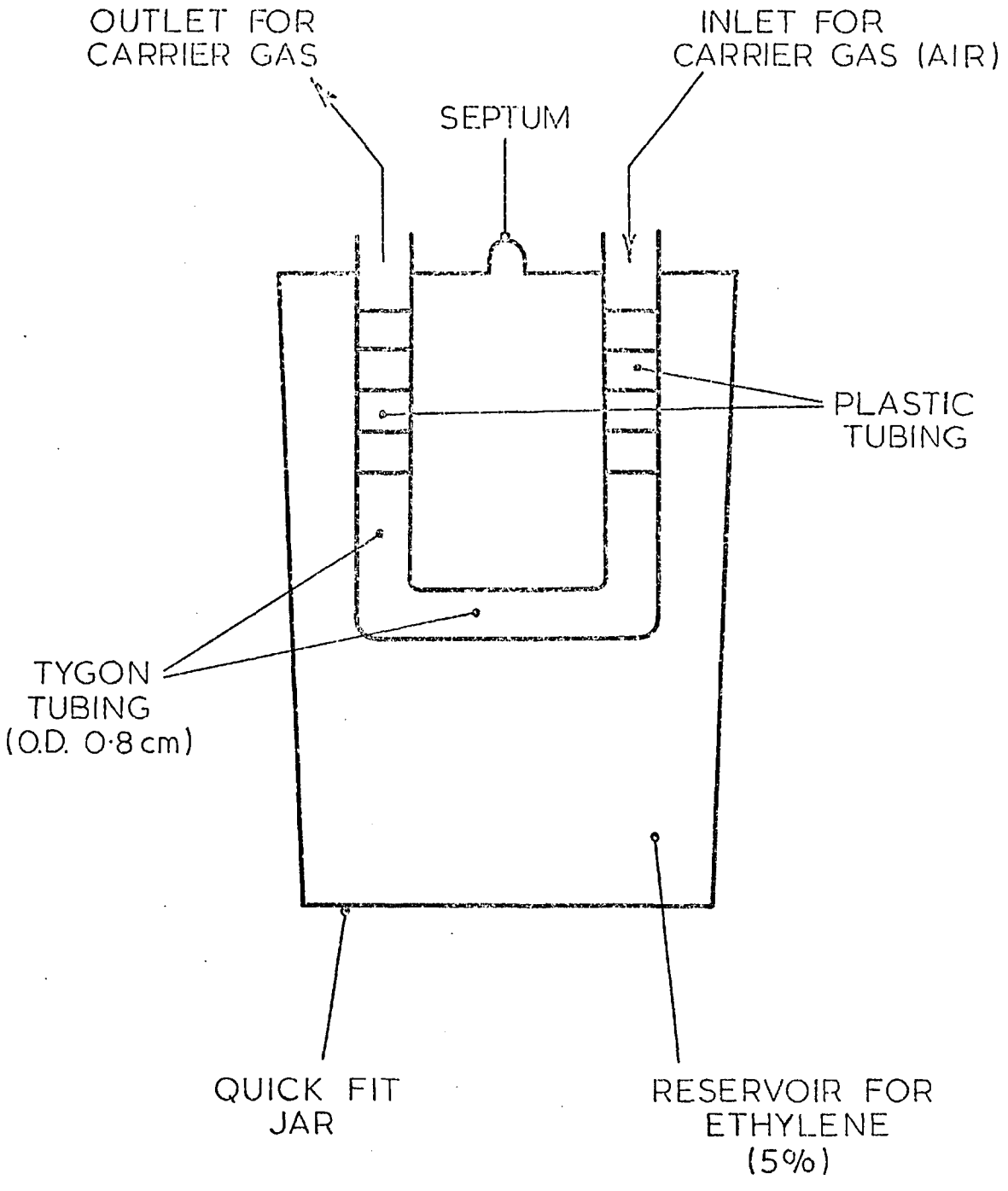


Figure 2: Ethylene dilution system.

Table 2

The combinations of gas flow rates and lengths of tygon tubing corresponding to the various ethylene concentrations required.

Required concentration of ethylene (ppm)	Length of tygon tubing (cm)	Flow rate of carrier gas (l/min)
0.01	1.0	4.0-4.5
0.03	3.0	2.0-2.5
0.10	10.0	0.9-1.0

The above dilutions and flow rates were constantly checked and variation in ethylene concentration is reported in the section for results. The reservoir was never recharged.

was weighed before each experiment and then three days after the onset of the climacteric. The mean weight loss per day was then computed. The mean loss per day for low and high humidities were about 0.6% and 0.1% respectively.

In later studies, water vapour pressure (WVP) was measured using an infrared vapour pressure analyser (type 225-NK3, serial number 4004, ADC, Hoddesdon, Herts, UK). This analyser was not used in previous studies because it was not available. The analyser had two ranges, range A 0--25 and range B 0-40 mb. Before water vapour pressure measurements were taken, the analyser was first calibrated. With the selector switched to ABS zero, and the range at A, all cells were purged with dry air and the zero set, using the instrument zero control. Air bubbled through water to full saturation at 20°C was then passed through the small analysis cells. After an equilibrium period, the fine gain control was adjusted to give a reading equal to the vapour pressure of the saturated air. The analyser was then ready for absolute measurement on range A and when necessary the B range was used. The WVP in each sealed jar containing a single fruit was measured by determining the water vapour concentration in both the influent and the effluent lines. The difference between the two readings was equivalent to the amount of water gained or lost by the fruit. For each humidity at least three readings were taken.

A major disadvantage in using the vapour pressure analyser was that there was a long time lag between readings from different humidities and hence only a few readings were taken from each treatment. The different humidities were controlled

as follows:- High humidity (wet air) was air humidified almost to full saturation, i.e. about 96 - 98% relative humidity, by bubbling it through water. Low humidity was either ambient air of about 60 - 70% relative humidity or air dried by passing it through two series of drying towers containing self-indicating silica gel. On the average this air was 4% relative humidity. There were two intermediate humidities which were mixtures of wet and dry air in the following preparations:-

1. two parts wet air and one part dry air.

2. two parts dry air and one part wet air.

Flow rates of component air of each mixture were metered and the 2:1 ratio corresponded to meter readings of 1.0 and 0.5 l/min, respectively. WVP readings were frequently taken using the water vapour analyser and these have been reported under results. Unless stated, in this thesis low and high humidities mean 96 - 98% and 60 - 70% relative humidity respectively.

2.2.3. Other methods

2.2.3.1. Vacuum infiltration

Fruits were treated with test solutions using methods described for apples by Scott and Wills (1977). Vacuum infiltration involved fruits in contact with water, with or without additions specified in the results for a total of 70 minutes which consisted of:- 2 minutes in contact with water

or solution before vacuum was applied, 5 minutes to reach the reduced pressure of 650 - 110 mm, 2 minutes at reduced pressure, 1 minute during the release of the partial vacuum and 60 minutes in contact with the solution while being removed from the vacuum tank. The uptake of solution for a single fruit was 0. 4 g which levelled off after 60 minutes. Where $CaCl_2$ was used it was expressed as a % (w/v) commercial product, not as % of calcium. Before salicylhydroxamic acid and benzyladenine were used for vacuum infiltration they were first dissolved in about 10 ml acetone.

2. 2. 3. 2. Pulp rupture force

Pulp rupture force was determined as described by New and Marriott (1974) for bananas. A compression force was applied to the pulp at a constant speed (1 cm/min). The probe was fitted with a force gauge on a motorised stand which elevated the sample at a constant speed. The stand was made at TPI workshop. The load cell (series G9, SANDOMONO Weston Controls Ltd. , Bognor, Sussex, UK) had a maxima range of 60 lbs. (W/kg.).

Transverse sections taken from the middle of the pulp (1 cm thick) were cut using a double bladed knife. The probe was inserted axially into the cortex avoiding vascular strands and the minimum force on the force distance recorded. Before measurements of pulp rupture force were taken the instrument was calibrated thus:- The range was set at 25 and the zero set and a force of 50 lb (static) was applied and the duo dial was manipulated to read 0. 500.

2. 2. 3. 3. Colour assessment

Colour was assessed using a standard colour chart developed by Loesecke (1949) for bananas. Colour was rated from dead green (1) to fully yellow (6).

2. 2. 3. 4. Determination of the duration of the preclimacteric

The preclimacteric phase was measured directly by CO_2 evolution or by inference from measurement of the pulp rupture force and colour (Peacock, 1966). CO_2 values for each fruit were plotted and specimen curves are shown in the section for results. Preclimacteric periods for each finger were then estimated graphically. For most studies an average of 11 fingers were sampled.

2. 2. 3. 5. Chilling injury

Chilling injury was assessed by visual inspection of the underpeel injury (Stover, 1972) in three green fruits after the various storage periods specified in the results (Table 4, 3. 2. 1.). Chilling injury was scored as nil (0), slight (1), moderate (2) and severe (3). Fruits which were already ripening (turning) were excluded from this assessment as they were more susceptible than unripe fruits to chilling damage (Simmonds, 1966).

CHAPTER 3

EFFECT OF STORAGE CONDITIONS ON THE PRECLIMACTERIC PERIOD OF PLANTAINS

3.1. Introduction

Plantains are frequently transported in bulk at a temperature of 25°C or above and fruits may take up to seven days in transit before reaching urban markets, which are often hundreds of miles from production areas. During this period, serious losses occur through ripening, softening and subsequent physical damage. In contrast, bananas are transported in refrigerated ships or lorries and transit ripening is minimised by control of both maturity at harvest and temperature during storage. The pattern of production of plantains by small farmers with minimal inputs will make refrigerated transportation and control of fruit maturity at harvest difficult to achieve with plantains destined for internal markets. However, the possibilities of manipulating plantain storage by control of temperature, by chemical treatment and by regulation of water loss (which is an unavoidable factor at ambient conditions in the tropics) are described. Plantains of cv. Apem were used in all studies and in selected experiments they were compared directly with bananas and/or with plantains of other cultivars.

3.2. Results

3. 2. 1. Measurement of the preclimacteric period

The duration of the preclimacteric was determined by either following rates of CO_2 evolution in single plantain fruits or colour change of the peel during ripening. The duration of the preclimacteric was calculated by extrapolation of CO_2 evolution at B in Figure 3 back to the basal rate, so that the preclimacteric period was taken as ending at A (i. e. 37 days). Investigations involving the determination of the preclimacteric period by colour change show that the first detectable change in peel colour occurred one day after the increase in CO_2 output (i. e. after the commencement of the climacteric), and the peel was uniformly yellow (i. e. colour score 6) three days later (Figure 4).

3. 2. 2. Effect of storage temperature

To measure the effect of temperature changes on the preclimacteric period, unripe fruits from three plantain cultivars were stored at four temperatures ranging from 6°C to 25°C. At measured intervals, after the commencement of storage, individual green fruits (i. e. three from each temperature) were removed and chilling damage assessed by visual inspection of the underpeel injury (brownish nectrotic tissues, see 2. 2. 3. 5.). In Tables 3 and 4 are results on the duration of the preclimacteric period and the chilling injury scores respectively. At 25°C, all three cultivars had preclimacteric periods less than one week. At 14°C, the preclimacteric period for each cultivar was double that at 25°C and similarly at 10°C the duration of the preclimacteric for each cultivar

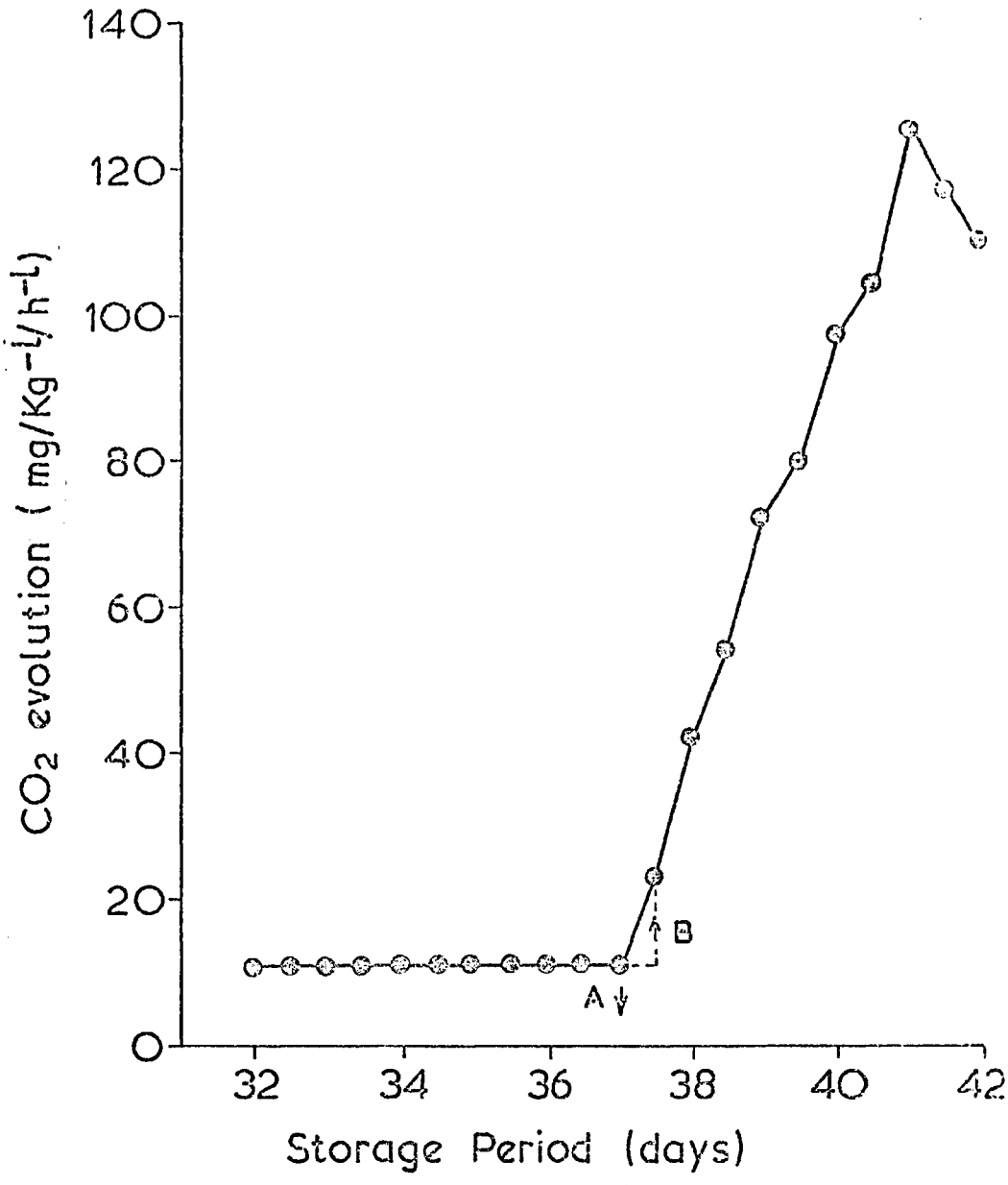


Figure 3: Calculation of the duration of the preclimacteric by following rates of CO_2 evolution of single plantain fingers. B, first observable increase in CO_2 production. A, end of preclimacteric period.

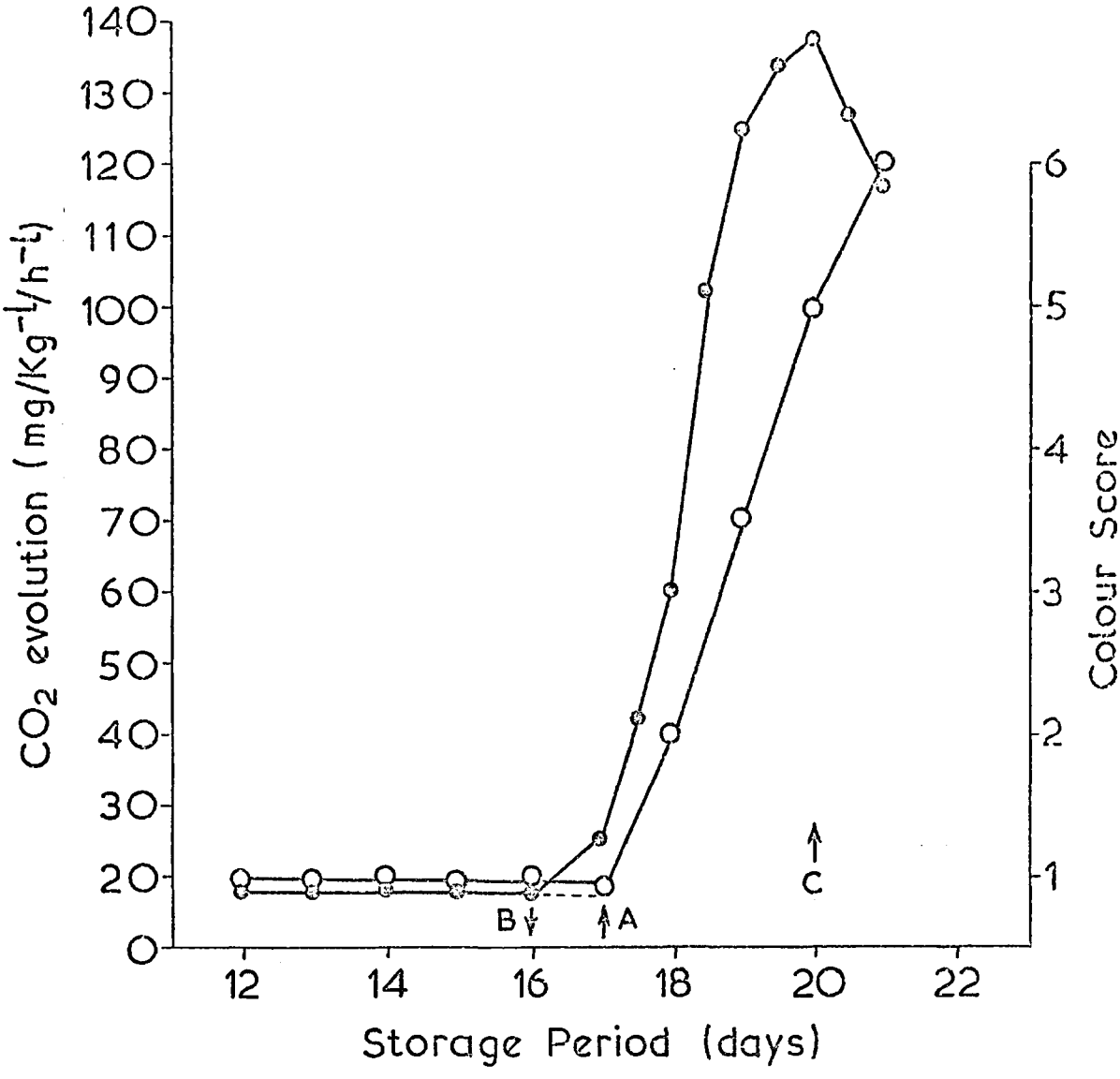


Figure 4: Relationship between rates of CO_2 evolution (⊶⊷) and peel colouration (○—○) of single plantain fingers. A, end of period prior to colour change; B, calculated preclimacteric period; C, climacteric peak. Fruits were scored until they became full yellow.

Table 3

The effect of temperature on the preclimacteric period of three plantain cultivars. Fruits were stored in bulk and the duration of the preclimacteric assessed by colour change. Data are means of observation for ten fruits.

Cultivars	Mean period (days) to reach full yellow colour at different temperatures			
	6° C	10° C	14°C	25°C
Apem	>28	20	10	5
Apantu	>28	18	8	4
Osa	>28	12	6	3

A chilling injury was apparent in all three cultivars within one week at 6° and 10°C.

Table 4

Chilling injury score for three cultivars of plantain stored at 6° and 10°C. Data are means for three fruits.

	Chilling injury score at different temperatures							
	6°C				10°C			
Storage period (days)	7	14	21	28	7	14	21	28
Apem	3	3	3	3	1-2	1-2	2	–[a]
Apantu	3	3	3	3	1-2	1-2	2	–
Osa	3	3	3	3	1-2	1-2	–	–

[a] – indicates fruits beginning to colour.

was double that at 14°C. At 6°C all three cultivars had pre-climacteric periods exceeding 28 days.

Chilling injury scores given in Table 3 do not include temperatures of 14°C and 25°C, as at these temperatures no chilling damage was observed. At 6°C all cultivars showed severe chilling damage (chilling score 3) within one week after the commencement of storage. In the third week, all fruits stored at 6°C became heavily pitted and when they were transferred to 25°C, they remained green and then turned black. Within two weeks after the commencement of storage at 10°C, chilling injury in all cultivars ranged from slight to moderate (score 1 to 2) and after three weeks of storage, fruits, mostly Apem and Apantu, had moderate chilling injury (score 2) and when these fruits were transferred to 25°C they remained green for some time and finally turned black.

3. 2. 3. Treatment with calcium chloride

The effect of calcium as aqueous $CaCl_2$ was assessed by vacuum infiltration of plantains with varying concentrations of calcium chloride up to 16% (w/v). Infiltration with water had no effect compared to undipped fruits and treatment with 4% to 16% $CaCl_2$ significantly increased the preclimacteric period, although treatment with 16% $CaCl_2$ also caused black spots on the peel (Table 5).

3. 2. 4. Treatment with salicylhydroxamic acid (SHAM)

To show whether the alternate oxidase associated with the

Table 5

The effect of aqueous $CaCl_2$ on the preclimacteric period of plantains. Fruits were stored in bulk and ripening assessed by colour change. Data are means of 20 fingers. The significance of differences between comparable means was tested using Duncan's multiple range test. Means followed by different letters were significantly different at the probability level $P = 0.05$.

Treatment		Mean period to reach full yellow colour (days)
Uninfiltrated		13.2 a
Water infiltrated		12.1 a
4% $CaCl_2$ (w/v)		16.0 b
8% $CaCl_2$ (w/v)		17.0 b
16% $CaCl_2$ (w/v)		15.2 b
	SE	1.36

respiratory chain was important in the control of the pre-climacteric, plantains and bananas were vacuum infiltrated with SHAM using two concentrations (10^{-3}M and 10^{-2}M). Neither concentration significantly (P=0.05) affected the duration of the preclimacteric period in plantains or in bananas (Table 6). Similarly, they did not affect pulp softening in plantains during ripening (Table 7).

3.2.5. Effect of Indole Acetic Acid (IAA) and Benzyladenine

In a single experiment, using only bananas, the effect of IAA and benzyladenine on the duration of the preclimacteric was examined by vacuum infiltration of green fruits with aqueous IAA (10^{-2}m) and benzyladenine (10^{-3}m). Both IAA and benzyladenine reduced the preclimacteric period, the former by 2.5 days and the latter by 1.4 days, when compared with untreated fruits (Table 8). The effect of these chemicals on plantains was not investigated.

3.2.6. Treatment with gibberellins (GA_3 and $GA_{4/7}$)

In three experiments, the effect of gibberellins on plantain storage was tested by dipping whole plantain fruits in gibberellic acid (GA_3) (10^{-4}M) and in gibberellin A_4 and A_7 ($GA_{4/7}$) (10^{-5}M). In the first experiment, both gibberellins extended the preclimacteric by one to two days when compared with untreated fruits (Table 9). Similarly, in the second and third experiments, gibberellins extended the preclimacteric period by five to seven days (Table 9).

Table 6

The effect of salicylhydroxamic acid (SHAM) on the preclimacteric period of plantains and bananas. Fruits were stored in a continuous flow system, using fifteen fruits per treatment. Ripening was assessed by colour change.

Treatment	Mean period to reach full colour (days)	
	Plantains	Bananas
Water infiltrated	21.8	16.6
SHAM (10^{-3}M)	23.2	15.6
SHAM (10^{-2}M)	23.0	15.9
SE	1.12	1.05

Table 7

The effect of SHAM on pulp softening in plantains. Data are means for five fruits.

Treatment	Mean pulp rupture force (kg) at different colour scores			
	2		6	
	Mean	SD	Mean	SD
Water infiltrated (control)	3.9	0.80	0.35	0.015
SHAM (10^{-3}M)	4.0	0.60	0.38	0.020
SHAM (10^{-2}M)	4.0	0.85	0.45	0.10

Table 8

The effect of growth regulators on the preclimacteric period of bananas. Fruits were stored in a continuous flow system, using fifteen fruits per treatment - and the duration of the preclimacteric assessed by colour change.

Treatment	Mean period to reach full yellow (days)
	Mean
Control (water infiltrated)	16.6
IAA (10^{-2}M)	14.1
Benzyladenine (10^{-3}M)	15.4
SE	1.15

Table 9

The effect of gibberellins on the preclimacteric period of plantains. Gibberellins were applied by dipping fruits for fifteen minutes. Fruits were stored in a continuous flow system using a total twenty fruits per treatment and ripening assessed by colour change.

Treatment	Mean period to reach full yellow colour (days)		
	Experiment 1	Experiment 2	Experiment 3
Undipped	10	15	15
Water	10	14	-
GA_3 (10^{-4}M)	11	20	-
$GA_{4/7}$ (10^{-5}M)	12	19	22

3. 2. 7. Effect of humidity

To determine the effects of water stress, individual fruit fingers of plantains and bananas were ventilated at a constant flow rate (4 l/h) with air at different humidities. In some experiments, water vapour concentration of influent and effluent air streams was measured. This work was repeated using other plantain cultivars, which were also compared with bananas. In Table 10 are data for preclimacteric period and water loss in two experiments with plantain (cv. Apem) with standard errors, which show that differences between low and high humidity were very significant. Data from these experiments and several others with the same cultivar, with two other cultivars (Apantu and Osa) and with bananas, are summarised in Table 11. Typically, an increase in the rate of weight loss by about 0. 5% per day was accompanied by a decrease in the preclimacteric period of about 60% (Table 11) in all the plantain cultivars. In bananas the preclimacteric period was reduced by about 40% for an increase in weight loss per day comparable with that for plantain cultivars.

A further experiment was carried out to examine changes in preclimacteric period of plantains at intermediate humidity using wet and dry air in the ratio 1:0; 2:1; 1:2; 0:1. The results are given in Figure 5. There was a large reduction in preclimacteric period when the mean weight loss was increased from 0. 05 to 0. 41% per day. There was a further substantial reduction in the preclimacteric period when the mean weight loss was increased to 0. 62% per day which seems to be the upper limit as a further increase to a value

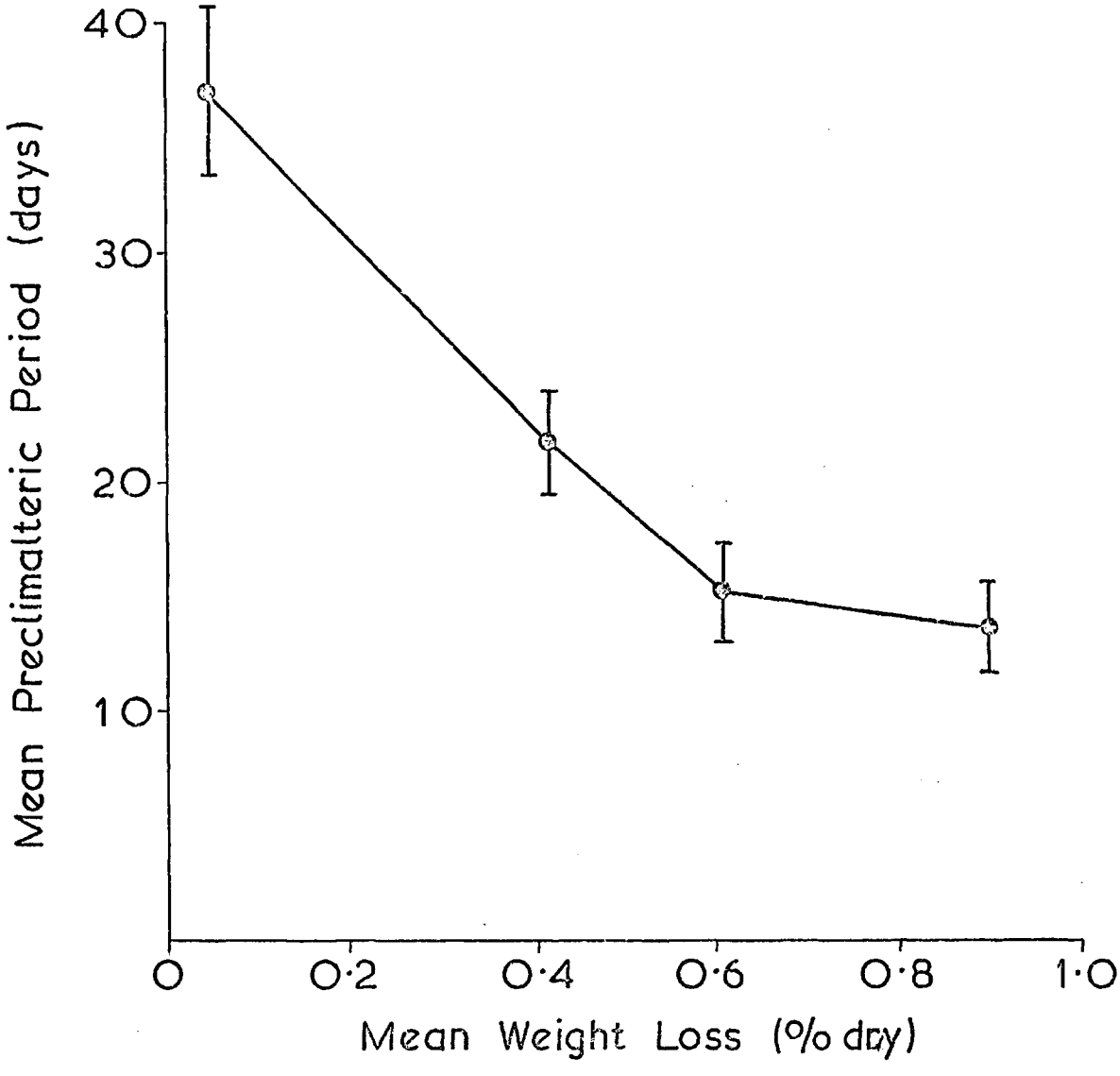


Figure 5: Relationship between weight loss and preclimacteric period of plantains, vertical bars denote twice the standard error (s. e.) for eleven fruits.

Table 10

The effect of humidity on the preclimacteric period of plantains. Data are means for eleven fruits. The significance of differences between means were tested using a 't' test.

Humidity	Preclimacteric Period (days)	Mean weight-loss (%/day)	
	Experiment 1	Mean	SE
Low humidity	8.4	0.56	0.012
High humidity	22.7	0.16	0.003
SE	0.51		
	Experiment 2	Mean	SE
Low humidity	12.5	0.60	0.015
High humidity	42.9	0.12	0.006
SE	0.97		

Table II

The effect of humidity on the preclimacteric period of three cultivars of plantain and on bananas. Data are means of observation on eleven fruits.

Mean preclimacteric period (days)		Reduction in preclimacteric period (%)	Δ rate of weight loss/day
High humidity	Low humidity		
Cv. Apem			
23	8	65	0.40
26	9	65	0.51
37	18	51	0.48
38	15	61	0.57
43	13	68	0.38
42	13	70	0.48
Cv. Apantu			
35	14	60	0.48
Cv. Osa			
39	14	64	0.48
Bananas			
22	15	32	0.53
25	14	44	0.57

Table 12

Effect of humidity on water loss and weight loss during plantain storage. a and b denote means for 11 and 6 fruits respectively. c and d are converted from water vapour concentration data - of ventilating air and air around fruits (flow rate 4 l/h). Fruit weight was about 200 g.

Humidity (%)				Water loss[b] (g/day)		Weight loss[a] (g/day)	
Influent Air[c]		Effluent Air[d]					
Mean	s. d.	Mean	s. d.	Mean	s. d.	Mean	s. d.
97.0	0.58	100	0.010	0.12	0.022	0.09	0.030
59.0	2.02	90.0	0.010	0.56	0.044	0.72	0.100
27	1.15	80.0	4.04	0.96	0.102	0.98	0.150
4.0	0.58	83.0	2.02	1.41	0.044	1.50	0.152

of 0.89% per day produced little effect. Table 12 shows that the data on water loss were comparable to those on weight loss (see discussion).

3.2.8. Effect of humidity and $GA_{4/7}$

To investigate whether $GA_{4/7}$ could increase the duration of the preclimacteric in plantains, under conditions favouring high weight loss, green fruits were vacuum infiltrated with this chemical using 10^{-5}M and then stored under two levels of humidity. In the two experiments, the separate effects of humidity without application and of $GA_{4/7}$ at high humidity were significant (Table 13) and were comparable with earlier results (Table 9). Moreover, when fruits were treated with $GA_{4/7}$ and stored under low humidity, their preclimacteric periods were not significantly different from untreated fruits stored under similar humidity.

3.3. Discussion

The end of the preclimacteric period was measured by extrapolation from CO_2 readings recorded automatically once each three hours (0.125 days). Thus the preclimacteric period was determined with a precision of $\pm$ 0.25 days. When measurement of the duration of the preclimacteric was based on changes in peel colouration, the first detectable change in colour was observed one day after CO_2 output increased and the interval between the start of the climacteric (ie CO_2 liberation) to when a fruit be came uniformly yellow was almost constant - and was about four days. Peacock (1966) observed a similar constant interval for bananas and used colour changes as an indicator of the end of the climacteric. Thus data in Figure 4 suggests that change

Table 13

The effect of humidity and gibberellins on the preclimacteric period of plantains. Data are means for eleven fruits. The significance between comparable means was tested using Duncan's multiple range test. Means followed by different letters significantly different at the probability level P = 0.05.

Humidity	Treatment	Mean preclimacteric (period (days)	Weight loss (%/day)	
	Experiment 1		Mean	SD
Low humidity	Nil	8.6 a	0.63	0.040
Low humidity	$GA_{4/7}$	8.3 a	0.62	0.030
High humidity	Nil	26.0 b	0.12	0.021
High humidity	$GA_{4/7}$	36.4 c	0.11	0.040
SE		0.65		
	Experiment 2		Mean	SD
Low humidity	Nil	13.4 a	0.38	0.030
Low humidity	$GA_{4/7}$	13.7 a	0.37	0.030
High humidity	Nil	40.8 b	0.06	0.021
High humidity	$GA_{4/7}$	53.4 c	0.06	0.020
SE		2.16		

in peel colour should be a reliable indicator of when a plantain fruit began its climacteric and consequently of the duration of its preclimacteric period.

The mean daily temperature in most plantain growing countries is usually between 24 and 28°C. It had been intended to carry out these investigations at 25°C but at this temperature, condensation occurred in the effluent (temperature) lines due to the lower temperature of the laboratory (normally about 22°C) and consequently 20°C (± 0.5) was used as the highest temperature readily obtainable. The short preclimacteric period observed at 25°C for the three cultivars is consistent with the short storage life of plantains at ambient temperature. The 100% increase in preclimacteric period observed in all cultivars when the storage temperature was reduced from 25° to 14°C and from 14° to 10°C is intermediate between increases previously reported by Hernandez (1973) and Guillemot (1976) for a similar reduction in the storage temperature of plantains. The chilling damage observed at 6° and 10°C and not at 14°C is consistent with previous observations that green (unripe) plantains are damaged by chilling at 10°C (Guillemot, 1976) and at 8°C (Hernandez, 1973), but are uninjured at 12°C. It would appear that the chilling injury range for plantains may be similar to the range (13° - 15°C) for bananas.

Vacuum infiltration of green plantains with calcium chloride solutions delayed the onset of the climacteric, but at the highest concentrations used (16% $CaCl_2$) fruits showed signs of skin injury, hence this concentration may be injurious. There is little published data on the use of calcium

chloride as a post harvest treatment in the storage of either plantains or bananas, though this compound has been tested as a pre and post harvest treatment in the storage of other fruits (Scott and Wills, 1975; 1977; Wills et al, (1977). The effect of calcium on the preclimacteric period of plantains observed in this thesis is similar to previous observations that calcium delays ripening in tomatoes (Wills et al, 1977) and in avocado (Tingwa and Young, 1974). Tingwa and Young (1974) found that in avocado treatment with calcium (0.1 m $CaCl_2$) depressed both respiration and ethylene production at the climacteric peak - and the magnitude of decrease was negatively correlated with high calcium levels in the fruit. The delay in ripening was, however, positively correlated with high calcium in fruits.

Salicyhdroxamic acid (SHAM), an inhibitor of the alternate oxidase pathway, was applied to see if it could delay the onset of the climacteric in plantains and bananas. Data in Table 6, however, suggest that the two concentrations used had little or no effect on the climacteric in plantains or bananas. For pears, Janes and Fenkel (1978) found that SHAM at concentrations of 10^{-3}M to 10^{-2}M did not delay the onset of the climacteric, but delayed pulp softening after its commencement. In plantains pulp softening was well co-ordinated with other ripening changes once the climacteric had commenced. No explanation was given why SHAM delayed softening in pears. Although SHAM might have inhibited the alternate oxidase in plantains and in bananas, the pulp softening which occurred in plantains suggests that the rise in repiratory climacteric may not reflect the operation of this enzyme. This

is consistent with observations by Theologis and Laties (1978) who show that in banana slices, the capacity of the cytochrome mediated respiratory pathway is sufficient to accommodate the climacteric and there is no evidence for the involvement of the alternate oxidase respiratory pathway (see 1.7.4. for more detail).

Banana fruits vacuum infiltrated with IAA and benzyladenine had shorter preclimacteric periods when compared with untreated fruits, though these differences were not significant ($P = 0.05$). Results with IAA are in agreement with those in a previous report that IAA accelerates ripening in whole banana fruits (Vendrell, 1969; Broughton and Wu, 1979). IAA has also been shown to stimulate ethylene production in bananas (Vendrell, 1970a). Similarly results with benzyladenine are in agreement with Blunden et al (1979) who showed 30 ppm kinetin, another form of cytokinin, accelerated ripening in bananas.

When plantains were dipped in gibberellin, their preclimacteric periods were increased, but treatment by dipping was less consistent and had less effect compared with that by vacuum infiltration (see Tables 9 and 13). Similarly, gibberellins, especially GA_3, have been shown to delay the onset of the climacteric in bananas (Vendrell, 1970b; Leonce, 1974; Kapoor and Turner, 1976) and $GA_{4/7}$ was more effective than GA_3 (Kapoor and Turner, 1976). In bananas, GA_3 (10^{-3}M) had little or no effect on the coordination of changes in respiration and ethylene production during the climacteric, but peel yellowing was delayed by two to five days after the commencement of the climacteric. Although fruits were ripe (as shown by both respiratory changes and soluble

solids), the peel was still green; the condition generally referred to as 'green ripe'. The effect of gibberellin on the co-ordination of ripening in plantains was further investigated and is reported in Chapter 4.

Factors which determine the rate of weight loss in fruits include temperature, relative humidity and rate of ventilation. In all the investigations reported in this thesis, both temperature and the rate of ventilation were kept constant so weight loss was largely dependent on the remaining factors. In a continuous flow system, the combined effects of relative humidity and vapour pressure deficit of the influent and effluent air streams establish an equilibrium humidity around fruits, which is at the same humidity as the effluent air. This equilibrium, which is affected by flow rates, determines the rate of desiccation.

Humidity data in Table 12 were converted from water vapour concentration, measured by an infrared water vapour analyser. Similarly, data on water loss were calculated from differences between water vapour concentration in the influent and effluent air. During the preclimacteric phase, the rate of transpiration is constant, but during the climacteric, transpiration rises rapidly then falls, parallel with respiratory changes (Wardlaw and Leonard, 1940). Consequently, measurements of vapour pressure were limited to the preclimacteric phase. Thus, data on water loss are more reliable indicators of water stress than those on weight loss. However, there was generally good agreement between data on loss of water and loss of weight (Table 12) and in the absence of water loss data

from many of the experiments, weight losses are reported and are believed to be an adequate indicator of water stress.

The magnitude of the effect of water stress observed with plantains is surprisingly large, representing a reduction in the preclimacteric period approaching three-fold for a change in weight loss from 0.12% - 0.16% per day (at high humidity) to 0.56%-0.60% per day (at low humidity). Weight losses of 0.5% per day are frequently exceeded under ambient conditions in the tropics, so that water stress must be a very important factor in determining the duration of the preclimacteric period of plantains during marketing and that of their tendency to ripen prior to sale. Data in Figure 5 shows that up to a weight loss of 0.62% per day, the duration of the preclimacteric period was directly related to weight loss, but this seemed to be the upper limit as an increase in weight of 0.89% per day produced little reduction in the duration of the preclimacteric period.

Thompson et al (1974) showed that low humidity accelerated ripening of plantains (Horse plantain, Horn type from Jamaica). Although their data did not distinguish between the effects of water stress on the preclimacteric period and on the rate of peel colouration, it is evident that where weight loss was almost 0.5% per day under stress conditions, peel colouration commenced after about 12 days rather than after 22 days when fruits were stored in moist coir dust.

Littmann (1972a) showed that water stress reduced the duration of the preclimacteric period of bananas and that the reduction

was directly related to the rate of water loss per day (up to 1.2% per day). If a linear relationship between weight losses and preclimacteric periods is assumed for data in Table 11 (i.e. up to 0.6% per day weight loss) in the presence of water stress, reductions in the preclimacteric periods for plantains (three cultivars) and bananas are 66% and 38% respectively for a weight loss of 0.5% per day. This means that for fruits with the same potential preclimacteric period, for 0.5% per day weight loss, the residual preclimacteric period of plantains will be about half of that for bananas.

Thus for unstressed control fruit with a preclimacteric period of 22 days and a weight loss of 0.5% per day, the residual preclimacteric period would be 8 and 15 days for plantains and bananas respectively, whereas Littmann (1972a) observed 19 days.

The effect of water stress over-rode the effect of gibberellin on the preclimacteric period, so that at low humidity the preclimacteric period was as low as the control (Table 13). Although it is probable that at intermediate levels of stress such as those tested in Figure 5, gibberellins would have some effect in extending the preclimacteric period, it is evident that gibberellin is not likely to be an effective means of extending the preclimacteric period under conditions favouring weight loss, such as occur at ambient conditions.

CHAPTER 4

THE CO-ORDINATION OF RIPENING CHANGES IN PLANTAINS

4.1. Introduction

In the preceding chapter, water stress and gibberellins were shown to affect the preclimacteric period in plantains in a manner qualitatively similar to that reported for bananas. In bananas, gibberellic acid (GA_3) has also been shown to delay peel yellowing after the commencement of the climacteric (Vendrell, 1970b), so that treated fruit become 'green-ripe'. In this chapter the effect of water stress and $GA_{4/7}$ on the co-ordination of changes in respiration, ethylene production, colour and texture changes during the climacteric in plantains will be described. These studies investigated whether storage conditions which affect duration of the preclimacteric also disturb the co-ordination of changes associated with the climacteric.

4.2. Results

4.2.1. Changes in respiration and endogenous ethylene production during the climacteric

To examine the effect of humidity and $GA_{4/7}$ on changes in respiration and ethylene production during the climacteric, Unripe fruits were vacuum infiltrated with $GA_{4/7}$ (10^{-5}M) and then continuously ventilated with air at two humidities. CO_2 and C_2H_4 evolution by single fingers were monitored

four - five days after the commencement of the climacteric. Results of this experiment, which are given in Table 14, indicate that both humidity and $GA_{4/7}$ had little or no effect on respiratory rates at the climacteric maximum or on the time taken to reach this from when the climacteric was initiated. The respiratory patterns of single fruits (Figure 6) also show that humidity and $GA_{4/7}$ did not affect normal climacteric patterns of respiration exhibited by plantains. Data on the separate effect of humidity on ethylene production (Figure 7) show that the co-ordination of changes in CO_2 and C_2H_4 evolution after the commencement of the climacteric was similar at the two humidities. Both at low and high high humidity, ethylene peaked 3.5 days before the climacteric peak, but the magnitude of ethylene production at low humidity was a little greater than that at high humidity.

4. 2. 2. Co-ordination of ripening changes

To examine the effect of $GA_{4/7}$ on the synchronisation of ripening in the peel and pulp, unripe plantains were vacuum infiltrated with $GA_{4/7}$ (10^{-5}M) and both treated and control (water infiltrated) fruits were continuously ventilated at constant rate (4 l/h) using air of two humidities. CO_2 evolution by each fruit was monitored until three days after the commencement of the climacteric. At measured intervals after the commencement of the climacteric, individual fruits were removed and pulp and peel separated. Peel colour was assessed using standard colour chart for bananas (see 2. 2. 3. 3.) and chlorophyll content determined by extraction and measurement of absorbance (see 2. 2. 1. 1.). Firmness

Table 14

Changes in respiratory activity of plantains during the climacteric. Data are means of observation of three fruits. The significance of differences between comparable means was tested using Duncan's multiple range test. Means followed by different letters were significantly different on the probability level P = 0.05.

Storage Humidity	$GA_{4/7}$ Treatment	Maximum rate of CO_2 production ($mg/kg^{-1}/h^{-1}$)	Time from onset to maximum of climacteric (days)
Low	-	128.3 a	2.8 a
Low	+	135.5 a	2.7 a
High	-	108.8 a	3.1 a
High	+	127.0 a	3.1 a
	SE	10.49	0.42

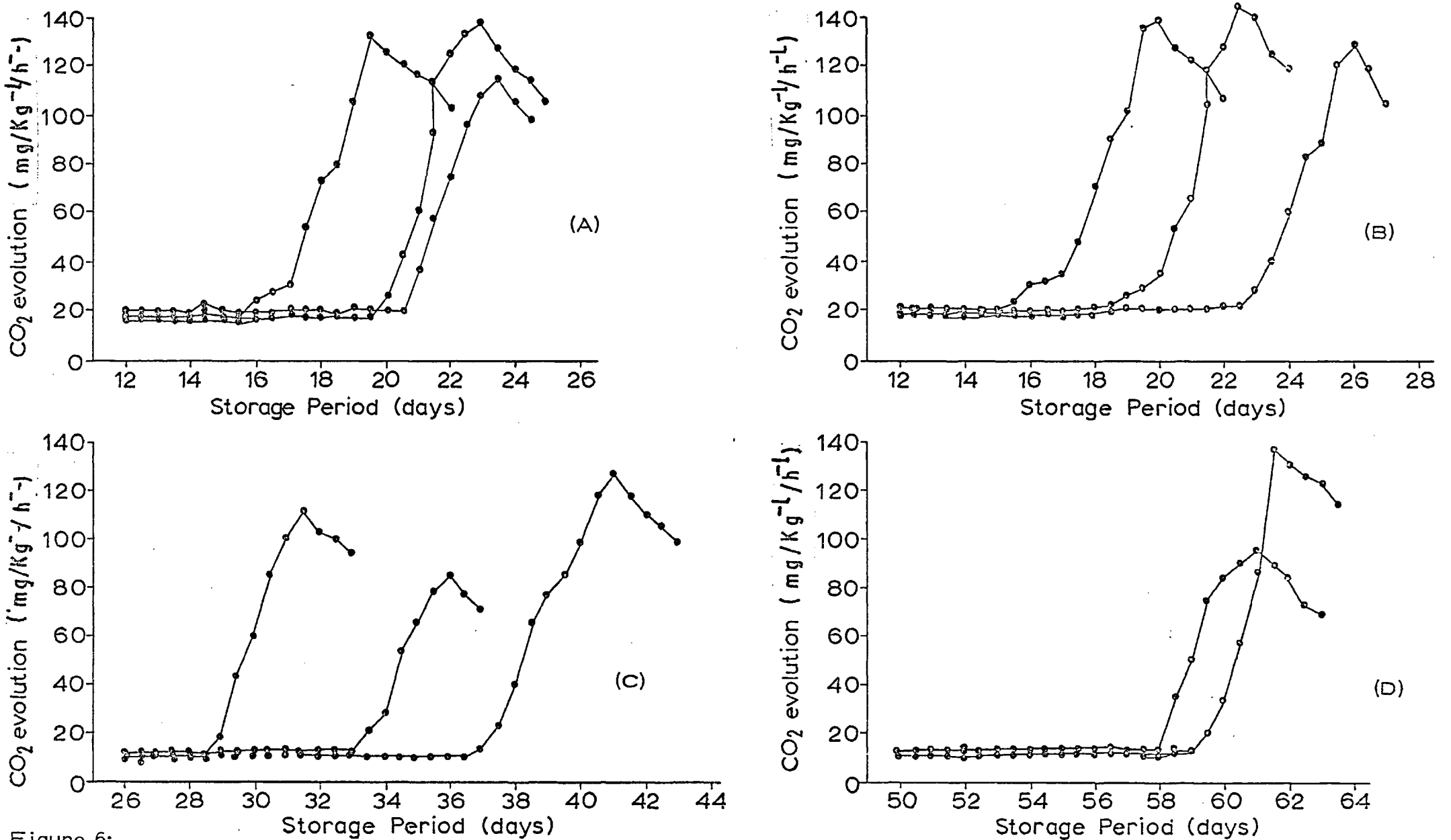


Figure 6:

Respiratory patterns of single plantain fruits treated with $GA_{4/7}$ and stored at two humidities. Untreated (A) and treated (B) stored at low humidity. Untreated (C) and treated (D) stored at high humidity.

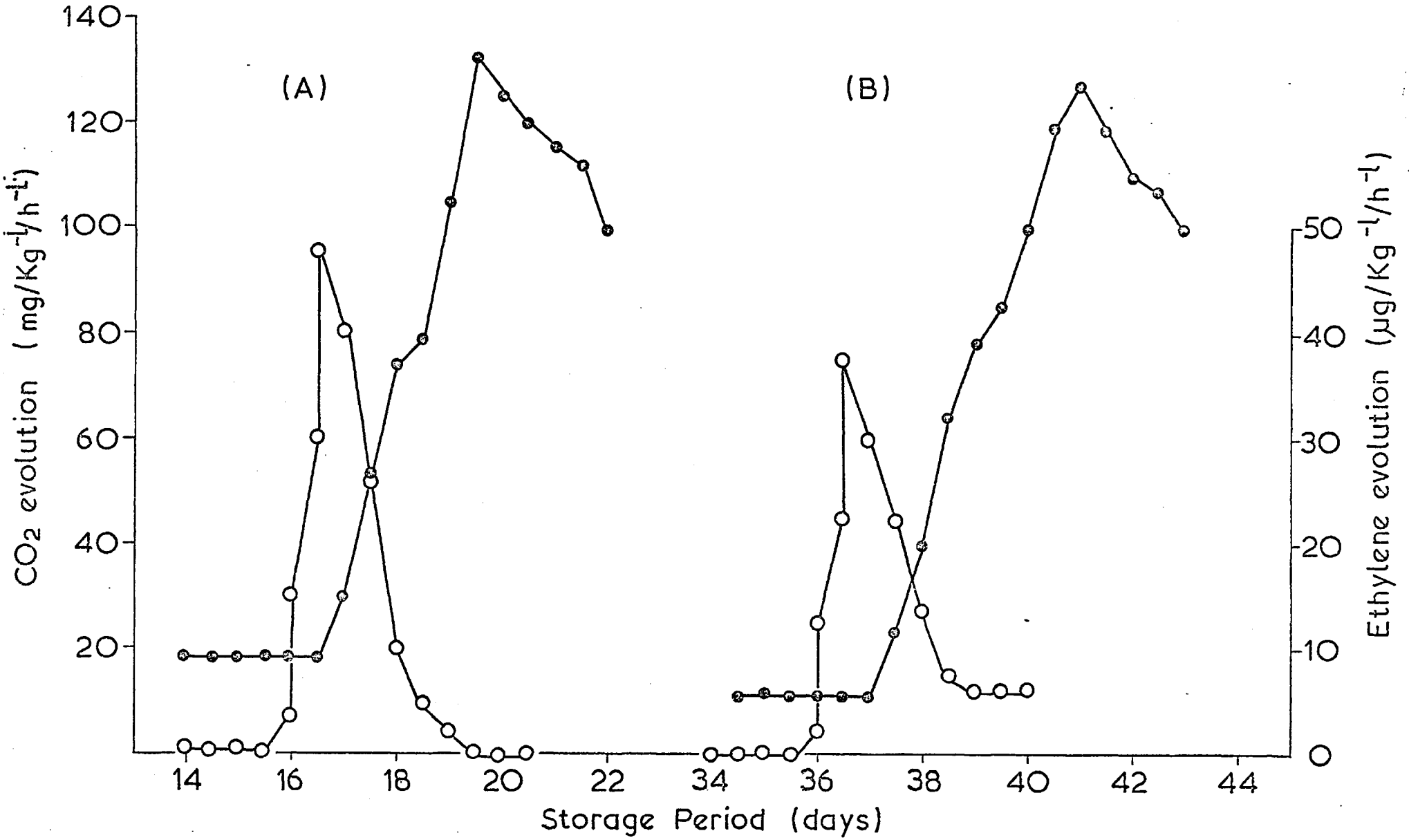


Figure 7: Rates of CO_2 (o—o) and C_2H_4 (o—o) evolution for single plantain fingers stored at two humidities. Low humidity (A) and at high humidity (B).

in the pulp was also determined. ACC levels in freeze-dried samples of the peel and the pulp of preclimacteric and climacteric fruits was determined by extraction and measurement of ethylene released by extracts (see 2. 2. 1.). In Figure 8, are summaries of results on changes in CO_2 evolution by the whole fruit, peel colour and chlorophyll and texture changes in the pulp. Treatment with $GA_{4/7}$ had little or no effect on peel colouration or on pulp softening or on CO_2 evolution by the whole fruit. Similarly, humidity had no effect on CO_2 evolution by the whole fruit or on peel colouration and pulp softening after two and three days from the commencement of the climacteric. After one day, peel colouration (as measured by chlorophyll degradation) and pulp softening were significantly delayed at low humidity when compared to changes at high humidity, this effect being evident both without and with gibberellin treatment. Data on the ACC content of climacteric plantains (Table 15) indicate that storage at low humidity increased the ACC level in the pulp, when compared with storage at high humidity, but the difference was only significant one day after the commencement of the climacteric. ACC was undetectable in the peel of climacteric fruits and in either the peel or the pulp of preclimacteric fruits stored at either humidity (i. e. less than 0. 03 n moles/g dry weight).

4. 3. Discussion

The literature on the effect of water stress on banana storage is conflicting, whereas Broughton and Wu (1979) found that water stress had no effect on the respiratory rates at the

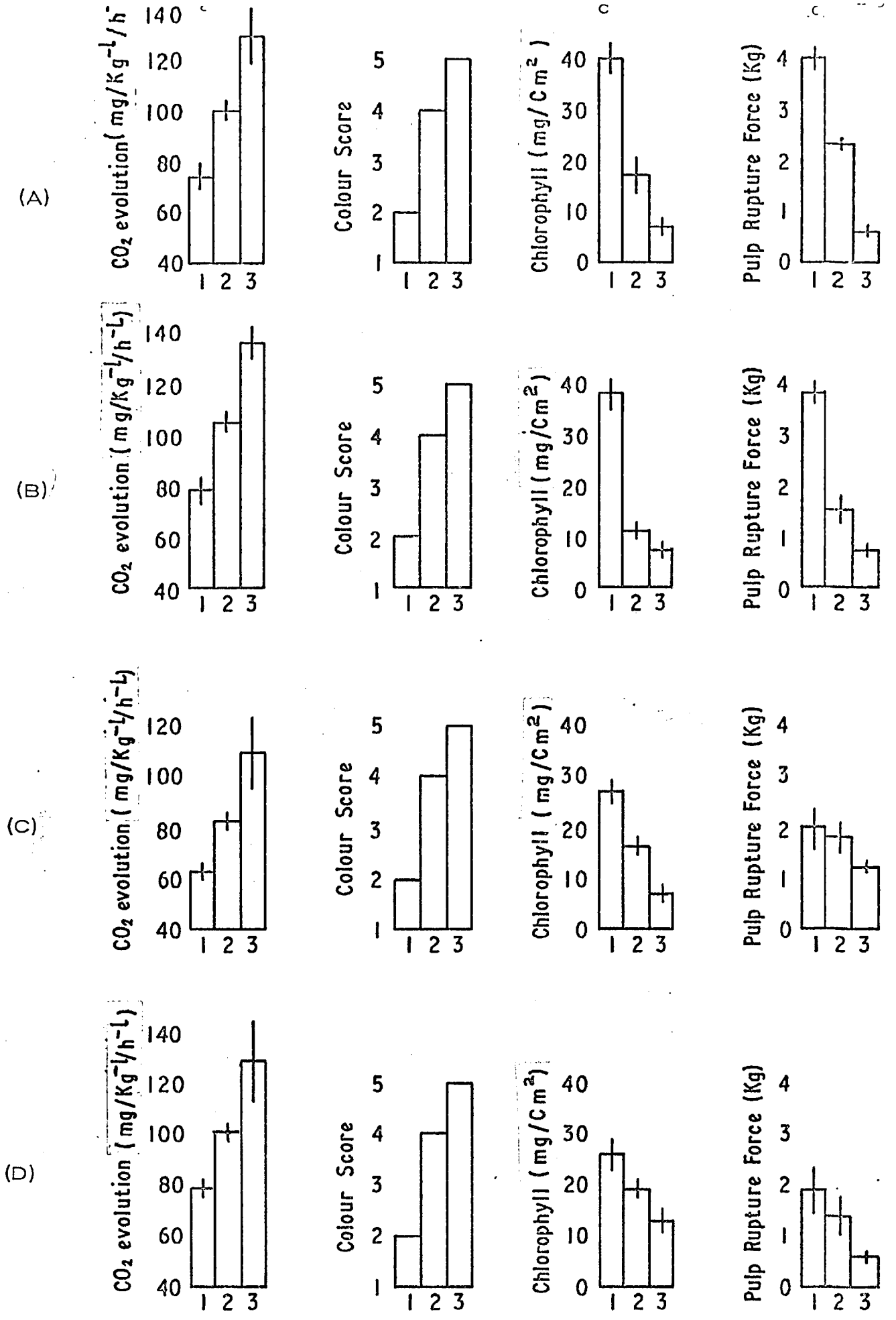


Figure 8: Effect of humidity and $GA_{4/7}$ on changes in respiration (a); colour (b); chlorophyll (c); and pulp rupture force (c) in plantains at 1, 2 and 3 days after the start of the climacteric. Untreated (A) and treated (B) with $GA_{4/7}$ and stored at low humidity. Untreated (C) and treated (D) and stored at high humidity. Vertical bars indicate twice the standard error of means for 3 fruits.

Table 15

Changes in ACC content of plantain pulp during the climacteric. Data are means of observation on three fruits. The mean efficiency conversion of ACC to ethylene was 54 ± 1.5%.

Period after commencement of the climacteric (days)	Low Humidity			High humidity		
	1	2	3	1	2	3
ACC content (n moles/g) ± SE	1.85	0.93	1.10	1.10	0.85	0.96
	0.056	0.061	0.031	0.031	0.086	0.058

climacteric peak, data from other studies (Littmann, 1972; Haard and Hultin, 1969; Lizana, 1975; Wardlaw and Leonard, 1940) show that water stress depressed it. To further complicate the picture, Haard and Hultin (1969) found that bananas stored at a humidity less than 80% did not undergo a respiratory climacteric (i.e. ripening was abnormal). Data of Lizana (1975) and Broughton and Wu (1979), however, show that bananas exhibited normal respiratory climacteric at humidities which were very close to zero.

There are also conflicting reports on ethylene production; while some workers (Lizana, 1975) found that water stress depressed the ethylene peak at the climacteric peak, others (Broughton and Wu, 1979) observed no effect of water stress.

These discrepancies, both in respiration and ethylene production, may have resulted from the different storage conditions and cultivars used by the various workers. In some of the experiments, bananas were apparently left in open rooms and after various storage periods ripening patterns of fruits sampled from the various rooms were studied. There was thus no control of humidity or flow rates of air used for ventilation. The effect of these and other factors on the rate of weight loss and consequently the effect of water stress have been described in Chapter 3 (3.3.). For instance, in a constant flow system, the equilibrium humidity of the effluent air is a function of both the humidity and the flow rate of the influent air. In some of the experiments different cultivars of bananas were used. For instance, Littmann (1972) used

Giant Cavendish, but Broughton and Wu (1979) used Gros Michel. While Gros Michel ripens readily, Cavendish cultivars do so with difficulty. Moreover, Gros Michel produces more ethylene than Giant Cavendish, thus the two cultivars may respond to ethylene produced under stress conditions differently. In order to resolve these conflicts in banana literature, a common methodology should be established and comparisons should be made between similar cultivars. The method used in the work on plantains reported in this thesis is similar to that used by Littmann (1972a,b,c) and Broughton and Wu (1979) for bananas and the results reported here are quantitatively similar to theirs in that in each case water stress accelerated the onset of ripening and a well defined climacteric was observed. Data of Wardlaw and Leonard (1940), of Haard and Hultin (1969) and of Lizana (1975) are completely contrary to these results in that the climacteric was both delayed and partially or completely supressed. The discrepancy cannot be resolved from published data and methods. All studies with plantains were replicated as were those of Littmann (1972a,b,c) and results were consistent with those of Littmann (1972a,b,c), Broughton and Wu (1969). The flow rate was as for Littmann and results were fully reported, other flow rates were not determined.

Data in Table 14 show that water stress has no effect on the magnitude of CO_2 evolved by plantain fruit at its climacteric peak and ~~in this~~ observations are similar to ~~that~~ by Broughton and Wu (1979).

The synchronisation of ripening of peel and of pulp in plant-

ains at 20°C is very close, so that the peel colouration (i.e. chlorophyll breakdown and subsequent peel colouring) and pulp softening, both commence within one day of the commencement of the respiratory climacteric. In bananas and in tomatoes treatment with GA_3 has been reported to delay peel colouration after the onset of the climacteric (Vendrell, 1970b; Babbitt et al, 1973). This delay in peel yellowing has been referred to as 'green-ripe' (see 3.4.). In plantains, $GA_{4/7}$ treatment (which has been found to be more effective in delaying ripening in bananas than GA_3) did not disturb the co-ordination of ripening, so that this chemical may be more practicable as a means of extending the storage life of plantains than it is for bananas. At low humidity both peel yellowing and pulp softening were delayed, relative to the onset of the climacteric, when compared to changes at high humidity, but this difference was only significant after one day not after two or three days.

ACC levels in the peel of climacteric plantains were measured to determine whether humidity affected the relative ethylene production in the two tissues during the climacteric. Unfortunately, ACC level in the peel was not measureable even during the climacteric. Hoffman and Yang (1980) showed that ACC level in the pulp of preclimacteric bananas stored at high humidity was undetectable and estimated it as less than 0.05 n moles/g. However, after the commencement of the climacteric, ACC levels in the pulp increased from an undetectable level to 6.5 n moles/g within four days after ripening and then dropped to 2 n moles/g on the sixth day. They compared the efficiency of ACC conversion to ethylene in purified banana extract with that of unpurified extract and reported 70 - 80% and 50 - 60%

for purified and unpurified extracts respectively. The conversion efficiency of 53±1.5% observed here for unpurified plantain extract is comparable to that reported for bananas.

Changes in the level of ACC are determined by the rate of ACC synthesis relative to its rate of utilisation (i.e. conversion to ethylene) or degradation. Thus, a decrease in the level of ACC during ripening will indicate that the rate of ethylene synthesis is greater than that of ACC synthesis. In plantains stored at high humidity, the level of ACC dropped from 1.10 u moles/g dry weight to 0.85, within two days after the onset of the climacteric, though on the third day there was a slight increase (Table 15). In bananas, ACC level remained high for four days and then decreased. The difference in the level of ACC might be attributed to the fact that plantains have much higher rates of ethylene production than bananas and this utilises more ACC.

CHAPTER 5

THE ROLE OF ETHYLENE IN THE EXTENSION OF THE PRECLIMACTERIC PERIOD OF PLANTAINS

5. 1. Introduction

Data previously reviewed in this thesis (1. 4. 1.) suggest that endogenously produced ethylene is a likely cause of the accelerated ripening and of the stimulation of respiration observed in preclimacteric bananas which have been subjected to water stress. Similarly, when preclimacteric bananas are continuously exposed to low ethylene concentration, the duration of their preclimacteric period is reduced and respiratory rate increased (Liu, 1976a). This study described here was carried out to determine whether or not endogenously produced ethylene could have a role in the effect of water stress on the duration of preclimacteric period of plantains (Chapter 3).

5. 2. Results

5. 2. 1. Changes in respiratory rates and in ethylene production in preclimacteric plantains

To investigate the mode of action of water stress in initiating ripening, green fruits were continuously ventilated with air of two humidities (low and high humidity). CO_2 evolution by each fruit was monitored until the onset of the climacteric. At measured intervals during the preclimacteric phase (i. e.

before the initiation of ripening), individual fruits (three from each humidity) were removed for internal ethylene measurements by cutting and expulsion of gas whilst immersed under water (2. 2. 2. 4.). Before each individual fruit was removed, a gas sample taken from the effluent air was analysed for ethylene using a gas chromatograph (2. 2. 2. 3.); if detectable ethylene production was observed, the fruit was not used for internal ethylene measurements. After cutting, a pulp sample was used for determination of refractometric solids (2. 2. 1. 2.). Data in Table 16 showing mean internal ethylene concentration suggest that low humidity raised it by two-fold. Data on refractometric solids show that all fruits sampled were still in their preclimacteric phase. In another experiment, a detailed study on changes in respiratory rates and in ethylene evolution was undertaken at intermediate humidity using mixtures of wet and dry air in the ratios 1:0, 2:1, 1:2, 0:1. Fruits were continuously held under these humidities and CO_2 evolution by each fruit monitored until the commencement of the climacteric. At measured intervals during the preclimacteric phase, rates of ethylene evolution by fruits stored at the different humidities were determined by trapping ethylene in the effluent gas stream (using three fruits from each humidity treatment). After a trapping period of 24 hours, ethylene was released from the ethylene mercuric perchlorate complex and the concentration determined (see 2. 2. 1. 4. for trapping method). In Table 17 are results showing the efficiency when known concentrations of ethylene were trapped. For the four ethylene concentrations

Table 16

Effect of water stress on internal ethylene concentration in preclimacteric plantains. Means are observation on nine fruits.

Humidity	Internal C_2H_4 (ppm)		Refractometric solids (%/fresh weight)	
	Mean	s. e.	Mean	s. d.
Low	0. 022	0. 0085	3. 8	0. 10
High	0. 010	0. 0036	3. 9	0. 17

Weight losses were 1. 20 and 0. 10 % per day for low and high humidity respectively.

trapped, the efficiency ranged from 75 - 81%, with a mean of 78%, this figure being used as a correction factor in calculation of ethylene production rates measured by trapping. In Table 18 are mean respiratory rates computed halfway through the preclimacteric period. Lower humidities (i.e. 27% and 4% relative humidity) significantly decreased the respiratory rates of preclimacteric fruits when compared to those at 97% relative humidity. Data in Figure 9 showing changes in respiratory rates at various stages of the preclimacteric phase indicate that respiratory rates increased sharply with decreased relative humidity. Thus at 97% relative humidity the respiratory rate was almost constant, i.e. 17 mg/kg^{-}/h^{-} throughout the preclimacteric phase, but at 4% relative humidity the respiratory rate increased by almost 12 mgCo_2/kg^{-}/h^{-} within five days.

To compare rates of ethylene production, means were computed through the first quarter and halfway through the preclimacteric period and the data in Table 18 shows that the rate of ethylene production was significantly increased by 4% and 27% relative humidity when compared with that for 59% or 97% relative humidity. Data on changes in ethylene production patterns in Figure 10 show that at relative humidity of 97% ethylene production was less than 0.01 µl/kg/h^{-} but it increased very sharply with decreased humidity, so that it was evident that with lower humidity (i.e. 27 and 4% relative humidity) ethylene production continuously increased. The preclimacteric periods given in Table 18 were for the experiment described in Chapter 3 (3.2.7.). (see discussion).

Table 17

Efficiency of ethylene trapping. Data are means of observation on three fruits.

Concentration of ethylene trapped (ppm)		Concentration of ethylene released after trapping (ppm)		Efficiency of trapping (%)	
Mean	S.E.	Mean	S.E.	Mean	S.E.
0.010	0.0013	0.008	0.0010	80.0	1.12
0.032	0.0051	0.024	0.0035	75.0	2.15
0.502	0.010	0.406	0.0131	80.9	1.65
1.101	0.030	0.871	0.0251	79.1	2.10

Table 18

Effect of water stress on respiratory rates and ethylene production by preclimacteric plantains. Data are means for three fruits. The significance of differences between comparable means was tested using Duncan's multiple range test. Means followed by different letters were significantly different at the probability level of P = 0.05.

Humidity (%)	Mean CO_2 evolution (mg/kg/h)	Mean rates of ethylene[e] production (µl/kg /h) at times preclimacteric period		Preclimacteric[f] period (days)
Influent Air		25%	50%	
		Preclimacteric Period		
93.0	17.3 a	0.005 a	0.0060 a	37.5
59.0	19.9 ab	0.0080 a	0.0098 a	21.1
27.0	21.7 b	0.0248 b	0.0338 b	15.2
4.0	39.8 c	0.0272 b	0.0352 b	13.9
SE	1.05	0.00281	0.00296 b	

e - for details see text.
f - see text.

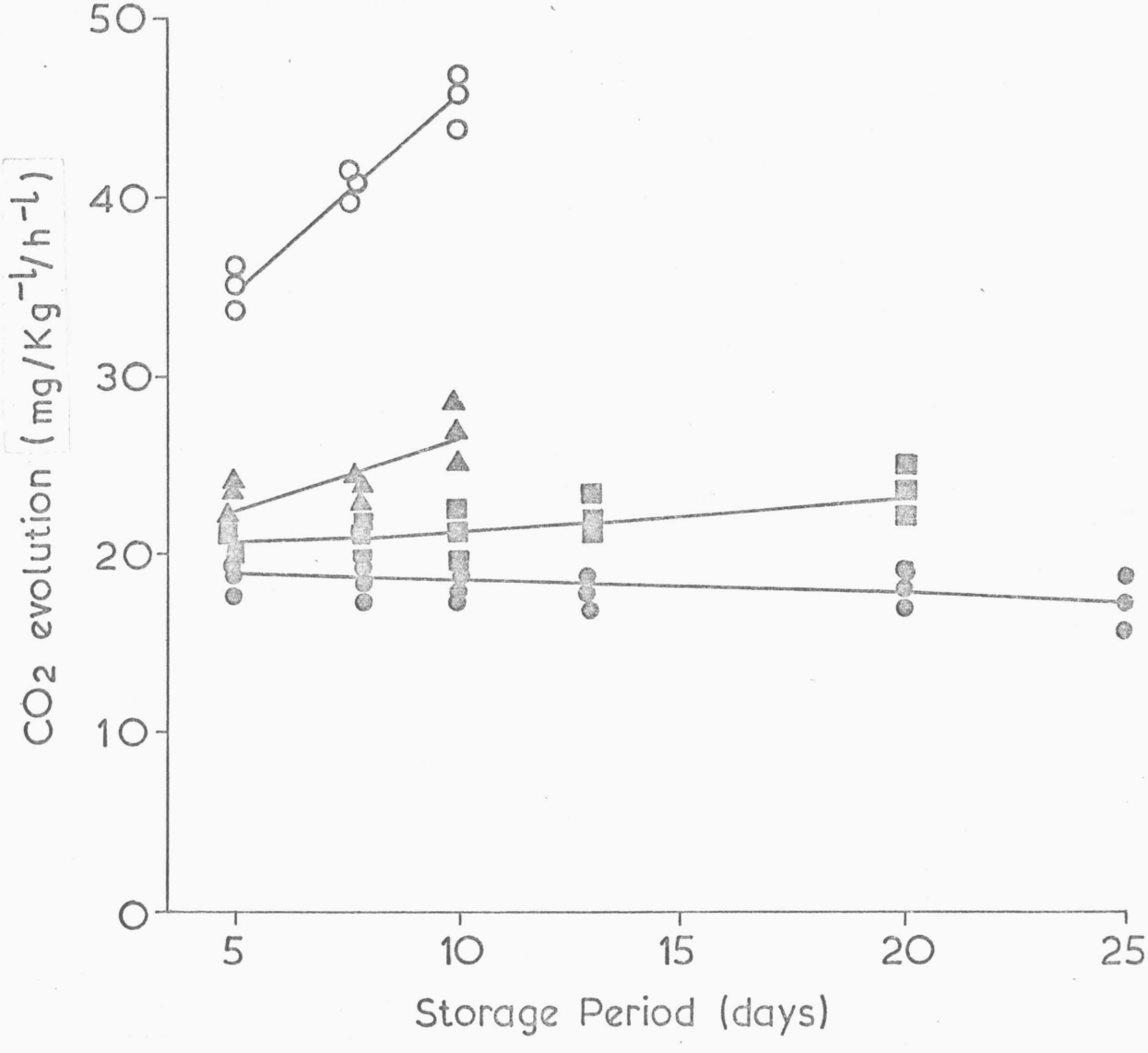


Figure 9: Effect of humidity on respiratory patterns in single plantain fingers.

Humidity: 97% ( )

59% ( )

27% ( )

and 4% ( )

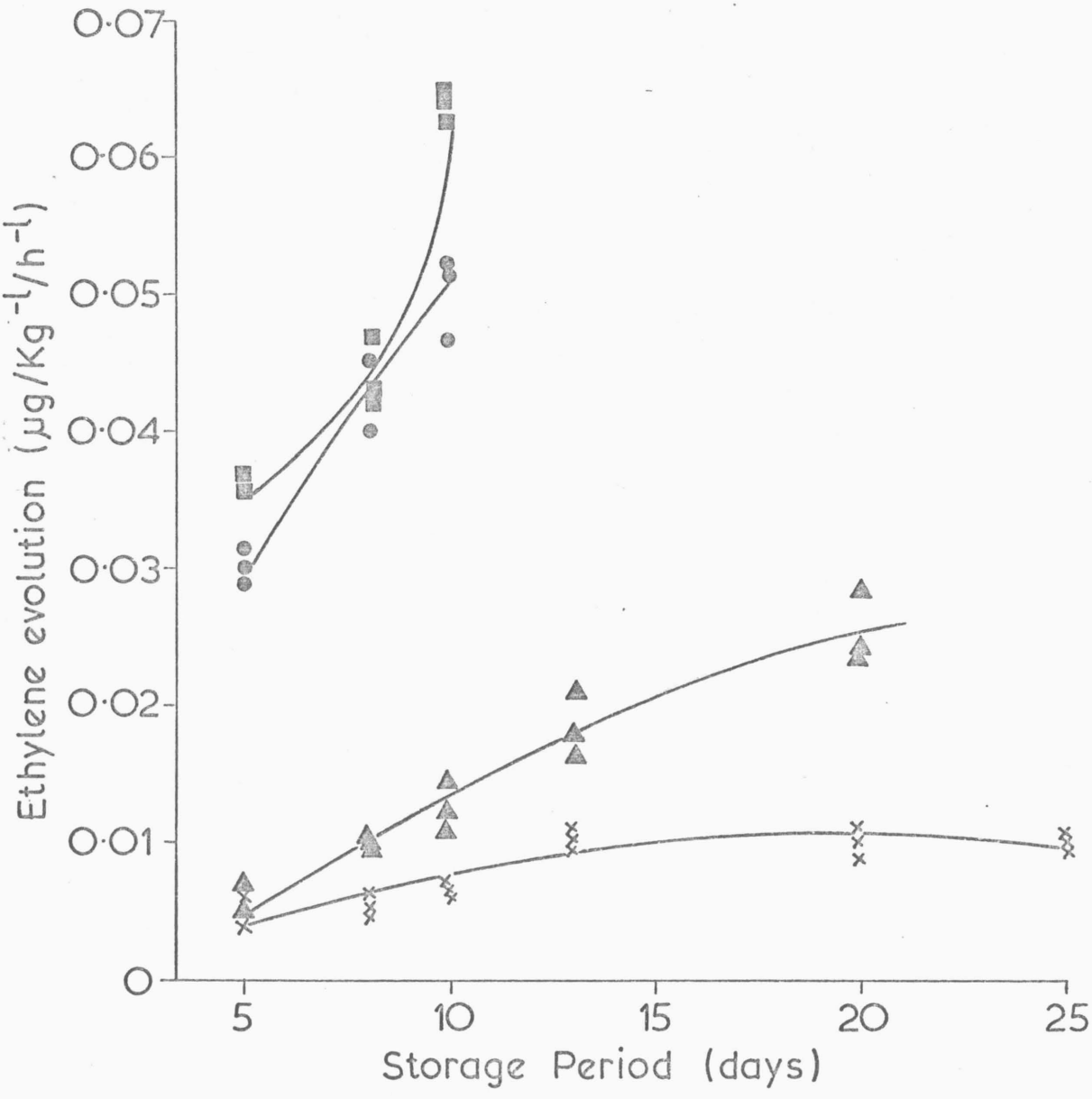


Figure 10: Effect of humidity on ethylene evolution patterns in single preclimacteric plantain fingers.

Humidity: 97% (✕—✕)

59% (▲—▲)

27% (●—●)

and 4% (■—■)

5. 2. 2. Effect of exogenous ethylene on the preclimacteric period and on respiratory rate

The effect of exogenous ethylene on the duration of the preclimacteric period and on respiratory rates during the preclimacteric phase was examined to show whether or not it could be correlated with that of stress ethylene. In two experiments, unripe plantains were exposed to ethylene, using different low concentrations maintained at a constant level. In the first experiment, fruits from two separate bunches were treated with three concentrations of ethylene ranging from 0. 101 to 2. 501 ppm, and in the second experiment fruits from a single bunch were treated with 0. 013 to 0. 105 ppm ethylene.

These concentrations were obtained by diluting ethylene using the dilution system already described (2. 2. 2. 5.). In order to exclude the effect of ethylene produced during water stress, all fruits were continuously ventilated with wet air, which caused minimal or no water stress. CO_2 evolution by each fruit was monitored until the commencement of the climacteric. Results from the two experiments are given in Table 19. In the first experiment all three ethylene concentrations significantly reduced the duration of the preclimacteric period and also increased the respiratory rates during the preclimacteric phase, when compared with untreated fruits. Differences between the two bunches in their response to ethylene was probably due to differences in bunch ages and maturity, since bunch B was more mature than A. Considering the results of the first and second

experiments it is evident that plantains respond to ethylene over a range from 0.01 - 0.5 ppm, but there is only a small further response when concentrations as high as 2.5 ppm are tested.

Data on mean respiratory rates calculated halfway through the preclimacteric period show that ethylene at concentrations from 0.039 to 2.501 ppm significantly increases the respiratory rate, when compared with that for untreated fruits. The effects of ethylene treatments on the kinetics of changes in respiratory rates given in Figure 11 show that five days after exposure to ethylene, the respiratory rates had increased and that this pattern was maintained throughout the preclimacteric period. The respiratory rates of untreated fruits were, however, almost constant.

5.3. Discussion

In the preclimacteric phase, plantains exhibit an increase in respiratory rates as the relative humidity was decreased. This increase in rate is different from the rise in respiratory climacteric, which is of a different magnitude. During the climacteric, the respiratory rate normally increases by almost 80 mg CO_2/kg/h within a 24 hour period. Whereas the largest change observed under water stress or with low concentration of ethylene applied to preclimacteric fruits was that in Figure 9 at 4% relative humidity, where the respiratory rate increased by 30 mg $CO_2/kg^{-1}/h^{-1}$ over a ten day period.

Table 19

Effect of exogenous ethylene on basal respiratory rates and on the preclimacteric period of plantains. In experiments one and two ethylene treatments commenced 5 and 3 days after harvesting fruits respectively. All fruits were continuously treated with the ethylene concentration stated. x means observation of eleven fruits; y means readings during the experimental period. The significance of differences between two comparable means was tested using Duncan's multiple range test. Means followed by different letters were significantly different at the probability of P = 0.05.

Ethylene treatment[y] (ppm)		Mean basal CO_2 production ($mg/kg^{-1}/h^{-1}$)		Mean preclimacteric period[x] (days)	
Mean	s.d.	Bunch A	Bunch B	Bunch A	Bunch B
		Experiment 1			
0.00	-	15.8 a	14.2 a	20.5 a	28.4 a
0.101	0.0101	25.1 b	24.4 b	-	2.4 b
0.502	0.0212	38.6 c	32.7 c	2.3 b	0.83 c
2.501	0.0180	48.0 d	46.5 d	1.2 c	0.75 c
	SE	3.15	4.15	1.02	0.98
		Experiment 2			
0.00	-	15.3 a		42.0 a	
0.013	0.0029	17.9 a		32.0 b	
0.039	0.0031	20.3 b		26.0 b	
0.105	0.0083	26.5 c		13.0 c	
	SE	1.92		1.05	

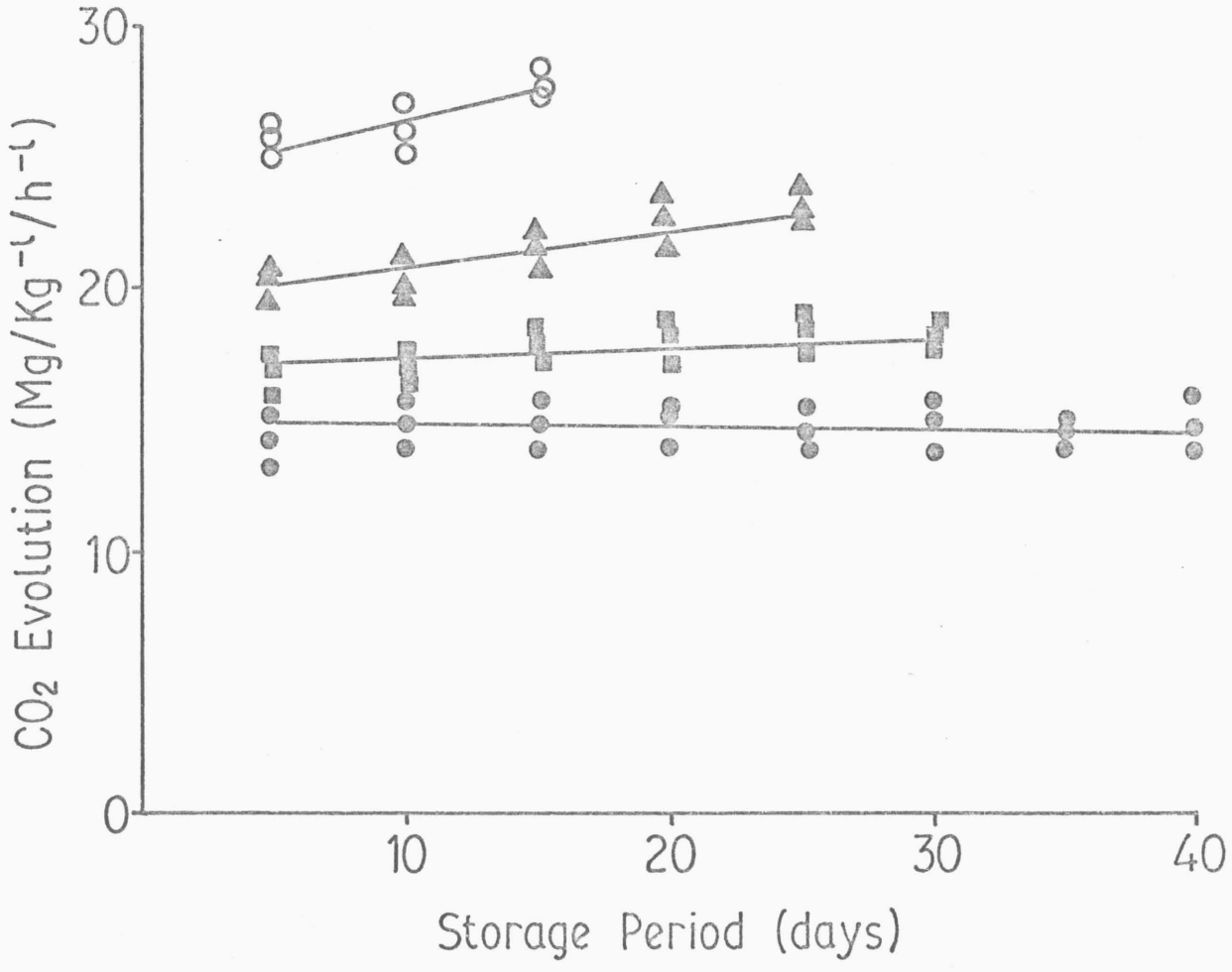


Figure 11: Effect of exogenous ethylene on respiratory patterns in single plantain fingers.

Control (⊸)
0.013 ppm (▪—▪)
0.039 ppm (△—△)
0.105 ppm (○—○)

Published data on the effect of water stress on the respiratory rates of preclimacteric bananas are conflicting, so that whereas Wardlaw and Leonard (1940), Lizana (1975) and Broughton and Wu (1979) found that water stress had no effect on the preclimacteric respiratory rate, Littmann (1972c) reported the reverse. Out of these accounts, in two of the investigations where no change was observed in respiratory rate, there was also a small increase (two days) in preclimacteric period in response to water stress (Wardlaw and Leonard, 1940; Lizana, 1975) and in the third one, the observed decrease in the preclimacteric period was about four days (Broughton and Wu, 1979), compared with 11 days reported by Littmann (1972a). Thus, the situation observed with plantains is similar to Littmann's observation in that there is both a substantial reduction in the preclimacteric period and an increase in respiratory rates prior to the onset of the climacteric.

The reason for the increase in the preclimacteric respiratory rates with water stress is not known, but it may suggest possible sites of respiratory control. If the control is exercised by the phosphate potentials as has been suggested by Laties (1967), increase in respiratory rates probably results from either the utilisation of energy or the uncoupling of oxidative phosphorylation. Bozhenco (1965) found that microelements (i.e. ions) which provided plants with resistance to water stress (i.e. drought) also increased ATP content in these plants and thus suggested that the ability of plants to withstand drought involves energy metabolism. On the other hand,

since water stress stimulates ethylene production and since this gas has been shown to increase the activity of adenosine triphosphatase in yeast micochondria (Olson and Spencer, 1968) Littmann (1972c) suggested that the uncoupling may be effected by this enzyme. ~~This is, however, detectable.~~ As water stress increases sucrose inversion in harvested sugar cane (Lauritgen and Balch, 1934), it is also possible that the increase in respiratory rates of plantains might have been caused by increased mobilisation of substrates. Present evidence, however, suggests that there is little or no change in the level of substrates (carbohydrate) in preclimacteric plantains (Marriott et al, 1980) to cause the increase in respiratory rates reported here.

Ethylene production by preclimacteric fruits was investigated firstly by measuring the internal concentration of the gas in fruits (with destructive sampling) and because each fruit sampled was destroyed, it was not possible to assess the rate of ethylene production in the same fruit up to the onset of the climacteric. The build up method described by Littmann (1972b) was also tried and before ethylene measurements were taken, fruits removed from stressing conditions. This method gave inconsistent readings as ethane from the laboratory interferred with ethylene peaks. ACC (precursor of ethylene) was also determined, but the level in preclimacteric fruit was not detectable. Similarly, Hoffman and Yang (1980) found that ACC content in preclimacteric bananas was not detectable. Finally, the trapping method was tried and this proved to be reliable, sensitive and permitted non-destructive measurements, without interferring with the stress imposed on the fruit.

Data on ethylene production are similar to that of Littmann (1972c) who showed that water stress increased ethylene production of preclimacteric bananas. His data showed that ethylene production for stressed fruits (i.e. at 13% relative humidity) was two-fold greater than that of unstressed fruits (i.e. 95% relative humidity). Ethylene production for stressed plantains (i.e. at 27% relative humidity) was four-fold greater than that for unstressed fruits (i.e. 97% relative humidity), so that although plantains were subjected to less stress than bananas, they still produced more ethylene. This difference in ethylene production might be due to physiological differences between plantains and bananas or to the different methods used for determining ethylene production discussed above.

Since the rate at which a fruit releases ethylene out of its tissues is related to the concentration within the tissues (Burg and Burg, 1961), it is possible to estimate the internal ethylene concentration in fruits from data on rates of ethylene produced or vice versa. Burg and Burg (1962) investigated the relationship between ethylene concentration within fruits and the rates of production of the gas for a wide range of fruits including bananas. For bananas, they found

that ethylene concentration within the fruit was related to the rates of production by a factor of 1.9 ppm for 1 µl/kg /h. Assuming that this factor is the same as for bananas, and that it does not vary at different levels of vapour pressure deficit, ethylene concentrations in the tissues of preclimacteric plantains corresponding to data on rates of ethylene production given in Table 18 are: for the first quarter of the preclimacteric period 0.010, 0.015, 0.047, 0.051 ppm at 97%, 58%, 27% and 4% relative humidity respectively; for halfway through the preclimacteric period 0.011, 0.019, 0.064, 0.067 ppm at 97%, 58%, 27% and 4% relative humidity respectively. Thus, as early as the first quarter of the preclimacteric period the difference between stressed and unstressed fruit should be about 0.04 ppm and this difference would tend to increase.

The sensitivity of plantains to ethylene was measured to investigate whether or not the effect of exogenous ethylene on the preclimacteric period was comparable to the effect of water stress (which generated endogenous ethylene) on the preclimacteric. 0.1 ppm ethylene decreased the preclimacteric period by 92% and 70% in the first and second experiments respectively, whereas 2.5 ppm and 0.013 ppm ethylene reduced the preclimacteric period by 96% and by 24% in the first and second experiments respectively. The difference in sensitivity (i.e. at 0.1 ppm ethylene) between the two experiments can be explained by considering published data on bananas. There are a lot of discrepancies in the literature on the sensitivity of bananas to ethylene. Both data reviewed by Palmer (1971) and that of Liu (1976a)

show that the sensitivity to ethylene increases as the fruit approaches its maturity, whereas Peacock (1972) observed that the sensitivity of bananas to ethylene decreased with increased maturity. He also observed that sensitivity to ethylene increased with time when ethylene was applied to fruits. Thus, when ethylene treatment was applied four and six days after harvesting fruits, the duration of the preclimacteric period was reduced by 4% and 16% respectively. In the first and in the second experiments described here, treatment with ethylene was commenced two and five days after the fruits were harvested respectively. Since the duration of the preclimacteric period of untreated fruits in the first experiment (Bunch B) was similar to that for untreated fruits in the second experiment, it is unlikely that the difference in sensitivity was caused by differences in maturity, but it is quite possible that it was caused wholly or in part by the differences in the time when ethylene was applied.

Comparing the effect of exogenous ethylene on the preclimacteric period in the second experiment (where fruits were treated with ethylene soon after harvest) with the effect of water stress on internal ethylene content and on preclimacteric period, it is evident that the effects of both exogenously applied and endogenously elevated ethylene concentration on the preclimacteric period are quantitatively similar Thus, 0.013 ppm of exogenously applied ethylene reduced the preclimacteric period by 24%, whereas water stress (which elevated endogenous ethylene concentration to 0.015-0.019 ppm) reduced the preclimacteric period by 45%. Similarly,

ethylene 0.039 - 0.105 ppm reduced the preclimacteric period by 60%; while water stress (i.e. 27% - 4% relative humidity, which elevated the endogenous ethylene concentrations to 0.047 - 0.064 ppm) reduced the preclimacteric period by 60%. Similarly, comparing the effect of exogenously applied ethylene on the basal respiratory rates with the effects of water stress on the basal respiratory rates, it is evident that the effects of both exogenously applied ethylene and endogenously elevated ethylene concentration have a similar effect on basal respiratory rates. Thus, the increase in basal respiratory rates of 17% caused by 0.013 exogenously applied ethylene is similar to that of 15% caused by water stress, which elevated endogenous ethylene concentration to 0.015 - 0.019 ppm. Similarly, exogenous ethylene of 0.039 ppm increased the preclimacteric respiratory rate by 33%, which is comparable to the increase of 25% in the basal respiratory rates caused by water stress (i.e. 27% relative humidity) which elevated ethylene concentration within the fruit to 0.047 - 0.064 ppm. However, the respiratory response of plantains to very high water stress (4% relative humidity) was notably different in that compared to a lower level of stress (27% relative humidity), there was little further reduction in the preclimacteric period (Figure 5, weight loss 0.62%/day) or further increase in ethylene production (Figure 10), but there was a large further increase in the respiratory rate, for which no explanation can be offered at present.

Water stress and application of exogenous ethylene differ in

that ethylene concentration increases with time in the former (Figure 10) and is constant in the latter, and this is reflected in that the rate of change in CO_2 production with time is much greater in the former (Figure 9) than the latter (Figure 11). Moreover, it is evident that the levels of ethylene generated within the first quarter of the preclimacteric phase are high enough to cause measureable effects both in respiratory rates and in preclimacteric period and that the effects are of a similar magnitude to changes which occur in water stress. It is thus probable that the very large effect of water stress on the preclimacteric period of plantains described in Chapter 3 is mediated wholly or largely through increases in synthesis of endogenous ethylene in response to this stress.

CHAPTER 6

GENERAL DISCUSSION

6.1. Effect of Water Stress

Of all the physiological factors tested in the present study, water stress had the largest effect on the preclimacteric period of plantains. Typically, for a weight loss of 0.5% per day, there was a reduction of 65% in the potential preclimacteric period, where the potential preclimacteric period is that of unstressed fruits. For the same weight loss, Littmann (1972a) reported a reduction of 15% in the potential preclimacteric period of bananas. Similarly, weight losses of 0.5% and 1.2% per day caused reductions of 30% and 36% in the potential preclimacteric period of pears and avocados respectively (Littmann, 1972a). In another study (Adato and Gazit, 1974) there was a reduction of 30% in the potential preclimacteric period of avocados for a weight loss of 2% per day.

Broughton and Guat (1979) found that water stress reduced the potential preclimacteric of custard apple (Annona squamosa L) by 50%, but gave no weight loss data. Comparing the data for plantains and bananas to those for avocados, pears and custard apple, it is evident that for similar weight losses, plantains are more sensitive to water stress than bananas and the other three fruits. Thus, when developing methods for the storage of plantains, it is necessary to control water stress prior to storage and during storage. Rapid movement of fruits from field to store is likely to be particularly critical

because the level of water stress will be particularly high during dehanding and bulking up of bunches. Storage trials show that the preclimacteric periods of both plantains (Hernandez, 1973; Guillemot, 1976; Ndubizu, 1976) and of bananas (Scott, 1971; Scott et al, 1970) can be considerably extended by storing fruits in sealed polythene bags, with or without ethylene absorbent. Flow-through investigations with bananas show that the level of oxygen in this treatment is more critical than that of carbon dioxide (McGlasson and Wills, 1972; Mapson and Robinson, 1966). Because of the large effect of water stress on the preclimacteric period of plantains, it is possible that when they are stored in a sealed system with an ethylene absorbent, the effect in reduction of water stress is comparable in importance to the effect of reduction of oxygen tension in extension of the preclimacteric phase. If this is true, then the rate of water loss could also be reduced by restriction of ventilation and this should be effective in extending plantain storage. However, since restriction of ventilation tends to encourage the build up of endogenous ethylene the use of this method along with an ethylene absorbent is likely to be more beneficial.

Investigations into the mechanism by which water stress reduced the preclimacteric period of plantains show that both the respiratory rates and ethylene production during the preclimacteric phase increased continuously when fruits are stored under stress conditions, and that the rate of increase was proportional to the level of stress. The only previous data on the effect of water stress on endogenous ethylene production is that of Littmann (1972c) on bananas. The high

effect observed with plantains compared with that observed by Littmann suggests that plantains are likely to be more suitable for studying the effect of water stress on the storage of climacteric fruits than bananas.

Although the role of ethylene alone was investigated, other growth hormones may also be involved in the accelerated ripening resulting from water stress. For instance, abscisic acid (ABA) has been shown to stimulate ripening (Addicott and Lyon, 1969) and water stress increases the level of this acid in tissues (Aharoni et al, 1977). It is therefore likely that the effect of water stress on the preclimacteric period of plantains involves changes in endogenous levels of ABA, as well as ethylene.

While there was evidence that relative to the onset of the climacteric, water stress slightly delayed the rate of the onset of the degradative processes both of chlorophyll breakdown in the peel and of softening in the pulp, the delay that was evident at 24 hours was not sustained at either 48 hours or 72 hours, so this was transient. Thus, in contrast to the situation in bananas, where water stress normally either decreases the rate and extent of the climacteric and/or prevents normal ripening (Haard and Hultin, 1969), the effect of water stress on plantains is apparently restricted to the preclimacteric period.

6.2. Effect of Gibberellins

Treatment with gibberellin has been shown to extend the pre-

climacteric period in various fruits such as tomatoes, bananas (Vendrell, 1970b; Babbitt et al, 1973; Dostal and Leopold, 1967). Apparently, after fruits were treated with gibberellin they were subsequently stored at high humidity. Although treatment with gibberellin extended the preclimacteric period of plantains, it only did so when storage was at high humidity; at low humidity it had no effect. Thus suggesting that gibberellin is not likely to be an effective means of extending the preclimacteric period of plantains under conditions favouring high weight loss, such as would occur at ambient conditions. Treatment with gibberellic acid (GA_3) has also been shown to retard peel colouration (ie synthesis of lycopene) and pulp softening in tomatoes after the commencement of the climacteric (Babbitt et al, 1973), without affecting the patterns of repiration. Similarly, GA_3 has been shown to retard peel colouration (chlorophyll breakdown), relative to the rise in respiration and pulp softening in bananas (Vendrell, 1970b). Results with plantains, however, show that treatment with gibberellin ($GA_{4/7}$) had no effect on either peel colouration or pulp softening. The synchronisation of ripening of peel and pulp of plantains is very close, so that both peel colouring and pulp softening commence within 24 hours after the commencement of the climacteric, thus treatments which disturb the co-ordination of these changes in other fruits may not have similar effects on plantains.

6.3. Effect of Ca^{2+}

Previous reports show that vacuum infiltration of various fruits with $CaCl_2$ solution delayed fruit ripening (Tingwa and Young, 1974; Wills et al, 1977). The results with plantains

are consistent with this finding. While plantains tolerated concentrations as high as 8% $CaCl_2$ (w/v) without any signs of skin injury, 6% $CaCl_2$ solution caused necrosis of the skin in Jonathan apples (Bengerth et al, 1972). 16% $CaCl_2$, however, caused a similar skin injury in plantains, and nearly exceeds the maximum safe concentration.

Infiltration of Ca^{2+} into fruits has been shown to reduce the respiratory rates (Faust and Shear, 1972; Tingwa and Young, 1974). High endogenous Ca^{2+} concentrations in avocado fruit also reduced the respiratory rates during the preclimacteric and climacteric phases (Tingwa and Young, 1974). In contrast, low Ca^{2+} concentration in apples increases the respiratory rates, and this increased respiration causes metabolic disorders such as internal breakdown and bitter pit (Faust and Shear, 1968). Consequently, the severity of these disorders could be minimised if $CaCl_2$ solution is applied to fruits in sufficiently high concentrations (Wills and Scott, 1977). Bain and Mercer (1973) also show that increased respiration resulting from low endogenous Ca^{2+} concentration in apples causes cellular disorganisation. As fruit ripening is usually accompanied by a progressive increase in cell permeability with alterations in the compartmentation of enzymes and substrates (Solomos and Laties, 1973) it is likely that Ca^{2+} delays ripening by preserving the cell membrane. Brady et al (1970b) showed that in bananas there is a steady increase in amino acid leakage and thus probably in membrane permeability as the preclimacteric phase progresses. Thus, the effect of Ca^{2+} in extension of the preclimacteric period of plantains may be exerted through an effect in stabilising the cell membrane.

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