Relationships between Respiration, Ethylene, and Aroma Production in Ripening Banana

J. B. Golding,[†] D. Shearer,[†] W. B. McGlasson,[†] and S. G. Wyllie*[,‡]

Centre for Horticulture and Plant Sciences and Centre for Biostructural and Biomolecular Research, University of Western Sydney, Hawkesbury, Richmond, NSW 2753, Australia

Mature green bananas were treated with the ethylene antagonist 1-methylcyclopropene (1-MCP) at intervals during the 24 h period after initiation of ripening with propylene. Following 1-MCP treatment, the fruits were ripened in either air or propylene while ethylene, carbon dioxide, and volatile production and composition were monitored at regular intervals. The application of 1-MCP significantly delayed and suppressed the onset and magnitude of fruit respiration and volatile production. The 1-MCP treatments also caused a quantitative change in the composition of the aroma volatiles, resulting in a substantial increase in the concentration of alcohols and a decrease in their related esters. The results showed that ethylene has a continuing role in integrating many of the biochemical processes that take place during the ripening of bananas.

Keywords: *Musa sp.; volatiles; esters; alcohols; ethylene; 1-MCP; CO2*

INTRODUCTION

Bananas (*Musa* sp.) are a typical climacteric fruit that exhibit a characteristic rise in ethylene production and respiration rate during ripening, followed some days later by the production of a range of characteristic aroma volatiles (Tressl and Jennings, 1972; Wyllie et al., 1998). Volatile production is an important quality characteristic of many fruits, which has been extensively studied (Schreier, 1984; Sanz et al., 1997); however, volatile biosynthesis during ripening is not fully understood. The use of 1-methylcyclopropene (1-MCP), an inhibitor of ethylene action, is a useful tool to help determine the role of ethylene in the development of aroma. The role of ethylene in initiating fruit ripening is well established (Lelievre et al., 1997); however, its mechanism of action, particularly in relation to subsequent volatile production, is not well understood. For example, it is not known which ripening events are dependent on ethylene action or how long they remain dependent. In a previous paper we examined these questions by treating bananas with 1-MCP at intervals of 6, 12, and 24 h following application of propylene, an active analogue of ethylene (Golding et al., 1998). We (Wyllie et al., 1998) reported that there were distinct quantitative differences in volatiles evolved by 1-MCP-treated fruit. This paper further explores the relationships between ethylene production, respiration, and the effects of 1-MCP treatment on the composition of the aroma volatiles evolved by ripening bananas.

MATERIALS AND METHODS

Fruit. Mature green bananas (*Musa* sp. [AAA group, Cavendish subgroup] cv. Williams) were obtained from a commercial grower at Coffs Harbour, New South Wales, Australia.

Fruits of closely related maturity (hands 2−4) were selected from one bunch. Control fruits from each hand took the same time to attain peak ethylene production ($P < 0.05$). Therefore, the data for the fruit from different hands were temporally adjusted, and the hands were treated as replicates. The experiment was a 4 × 2 factorial with the experimental design being a randomized complete block design with three replicates.

Individual bananas were dipped in thiabendazole solution (22.5 g L^{-1}) for 4 min and air-dried. All experiments were conducted at 20 °C.

The fruits were individually sealed in respiration containers and ventilated with humidified air at ∼1 L h^{-1} for 18 h. Ethylene and carbon dioxide were measured to ensure that the fruits were preclimacteric (Golding et al., 1998). To initiate ripening, fruit were ventilated with water-saturated air containing propylene (500 μL L^{-1}). Propylene is an active analogue of ethylene that enables the measurement of endogenous ethylene production during the ensuing increase in respiration (McMurchie et al., 1972). This concentration of propylene is equivalent to ∼5 μL L^{-1} of ethylene (Burg and Burg, 1967), an amount previously shown to give optimum advancement of endogenous ethylene production (McMurchie et al., 1972).

When the bananas were considered to be fully ripe, that is, when the peel was fully yellow with lightly flecked and brown areas (color stage 7) (CSIRO, 1971), all measurements on the individual bananas were terminated.

1-MCP Synthesis and Application. 1-MCP was synthesized according to the method of Magid et al. (1971) and stored in a solution of methyl allyl chloride under nitrogen at −20 °C. Fruits were sealed in 6 L containers and fumigated with 1-MCP (45 μL L^{-1}) for 1 h at 6, 12, or 24 h after propylene treatment (HAPT). Aliquots of the 1-MCP solution were injected through a rubber port into the sealed containers onto a glass filter disk and allowed to volatilize (Golding et al., 1998). Because previous experiments showed that the byproducts associated with release of 1-MCP (mainly ethyl ether and cyclohexane) had no physiological or biochemical effects on banana fruit ripening (unpublished data), a solvent control treatment was omitted from the experiments reported here.

Following treatment, bananas were sealed individually in 2 L respiration containers and ventilated with measured flows of humidified air (∼1 L h^{-1}) with or without propylene (500 μL L^{-1}). Control fruits were not treated with 1-MCP.

* Author to whom correspondence should be addressed (telephone +61 2 4570 1762; fax +61 2 4570 1671; e-mail g.wyllie@uws.edu.au).

[†] Centre for Horticulture and Plant Sciences.

[‡] Centre for Biostructural and Biomolecular Research.

10.1021/jf980906c CCC: $18.00
Published on Web 03/25/1999

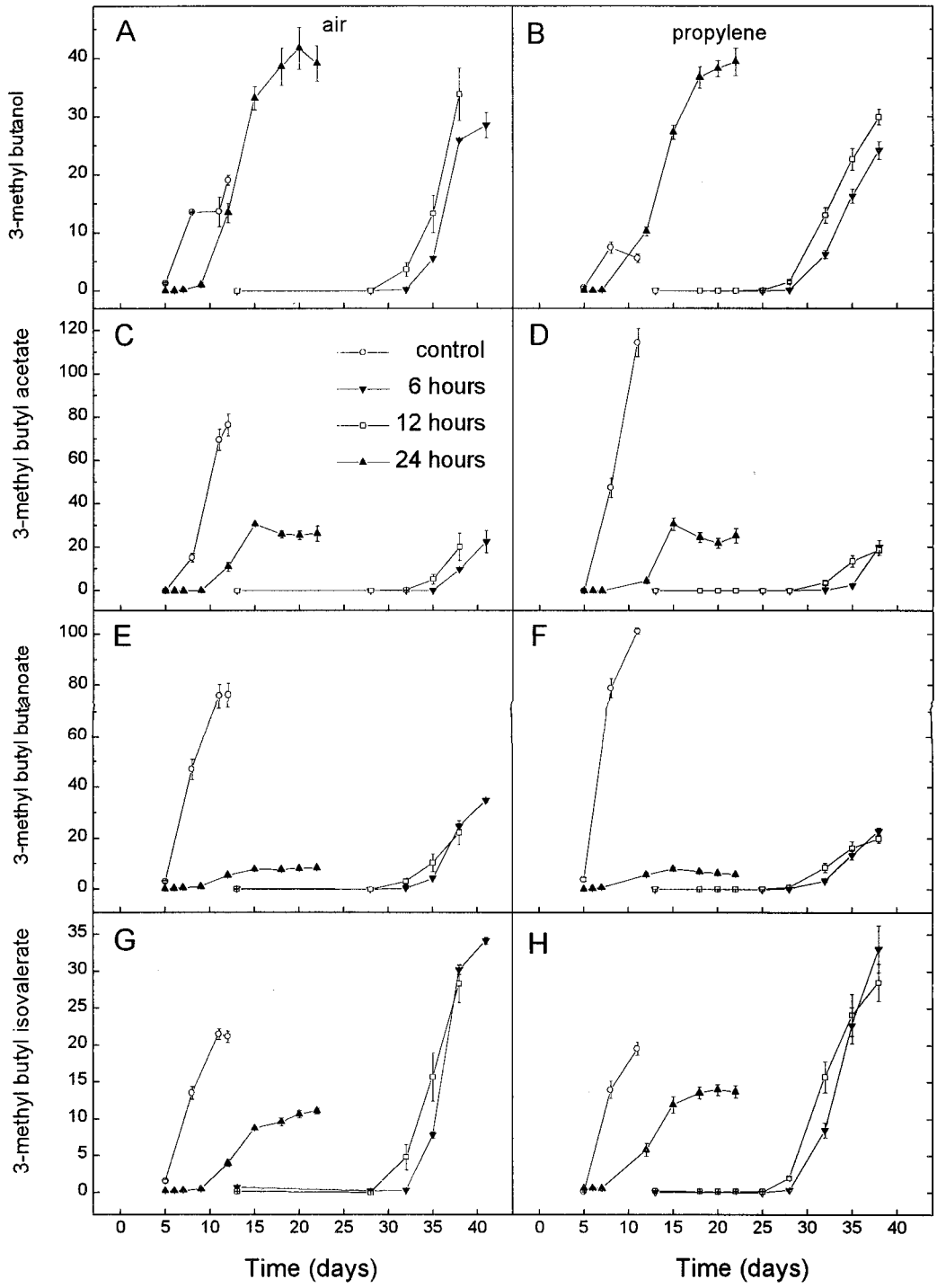

Figure 1. Rates of production (μg L^{-1} kg^{-1}) of 3-methylbutanol, 3-methylbutyl acetate, 3-methylbutyl butanoate, and 3-methylbutyl isovalerate by fruit treated with 1-MCP at 6, 12, and 24 HAPT. The control fruit had no 1-MCP. Following 1-MCP treatment, the fruits were either ventilated with humidified air (A, C, E, G) or treated continuously with propylene (B, D, F, H). The bars show the standard errors (SEs) of the means (n = 3). When absent, the SE bars fall within the dimensions of the symbol.

Volatile Analysis. *Volatile Sampling.* Glass gas chromatograph injector liners packed with Tenax TA (40 mg) were used as volatile collection traps. These traps were connected to the individual respiration chamber outlets, and the effluent was collected until a known volume of headspace (200−500 mL) had passed through each trap.

Volatile Analysis. Each trap was thermally desorbed with a programmable temperature vaporizer (OPTIC 1, Ai, Cambridge) connected to a Hewlett-Packard 5890A gas chromatograph fitted with an FID. Analysis was carried out on a BP-1 fused silica capillary column (SGE), 25 m × 0.22 mm i.d. × 1.0 μm film thickness. The split/splitless injector had a split ratio of 20:1.

The analysis was initiated by programming the injector temperature from 40 to 220 °C at 16 °C s^{-1}, where it remained for the rest of the analysis. The column was maintained at 40 °C for 5 min and then programmed at 10 °C min^{-1} to 200 °C. The FID detector was maintained at 220 °C. Data were collected using a Hewlett-Packard Chemstation 3365 data processing package.

Volatiles were identified by comparison with the retention times of authentic standards (Sigma Chemical Co.) and Kovats indices and confirmed by gas chromatography/mass spectrometry.

Rates of Volatile Production. The rates of volatile production were calculated from the integrated area of each of the selected

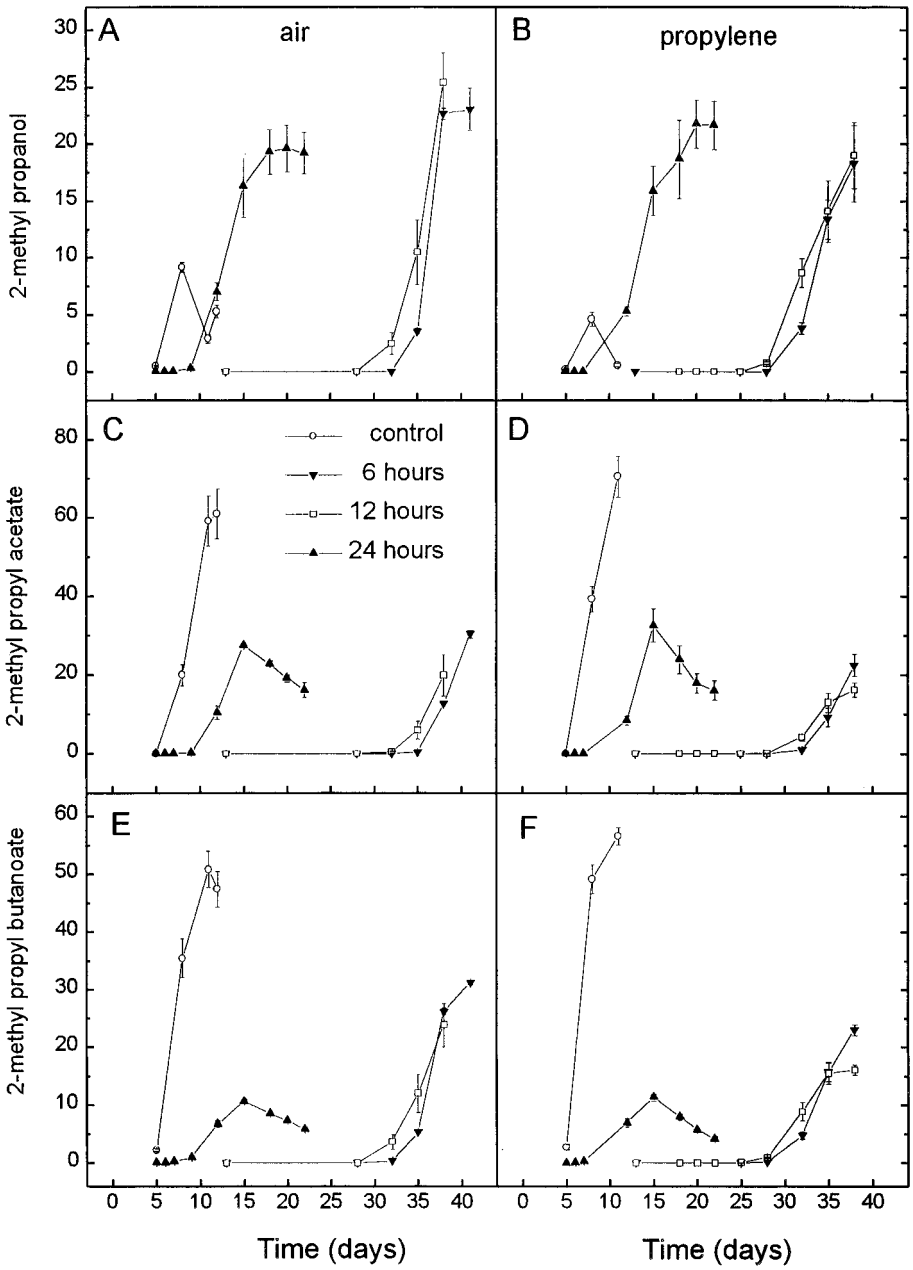

Figure 2. Rates of production (μg L^{-1} kg^{-1}) of 2-methylpropanol, 2-methylpropyl acetate, and 2-methylpropyl butanoate by fruit treated with 1-MCP at 6, 12, and 24 HAPT. The control fruit had no 1-MCP. Following 1-MCP treatment, the fruits were either ventilated with humidified air (A, C, E) or treated continuously with propylene (B, D, F). The bars show the SEs of the means ($n = 3$). When absent, the SE bars fall within the dimensions of the symbol.

peaks. This was used to calculate the amount (micrograms) each peak represented by comparison with injections of external standard (2-methylpropyl acetate) of known concentration. All compounds were assumed to have a relative response factor of 1.0. These figures together with the volume of headspace collected and the weight of the fruit sample gave the production rate for each compound (μg L^{-1} kg^{-1}). Total volatile production was calculated by accumulation of these data.

RESULTS AND DISCUSSION

Relationships between Ethylene Production, Respiration, and Total Volatile Production. In our previous paper (Golding et al., 1998) we reported the changes engendered in a range of banana physiological characteristics following the application of the ethylene antagonist 1-MCP. The 1-MCP treatment times were selected to precede and coincide with the ethylene climacteric of bananas for which ripening had been initiated by propylene application.

Application of 1-MCP at 6 and 12 HAPT resulted in delays in the onset of both ethylene production and respiration of between 27 and 24 days, respectively, for air and 20 and 19 days, respectively, for a propylene environment. These large delays suggest that the ethylene-dependent events which are part of the normal ripening biochemistry are suppressed by the application of 1-MCP at 6 and 12 HAPT and are unable to resume until ethylene sensitivity has been regained. However, ethylene-independent events should still proceed normally. As a result, a different biochemical environment will be present within the fruit when ripening subsequently does take place in these 1-MCP-treated bananas.

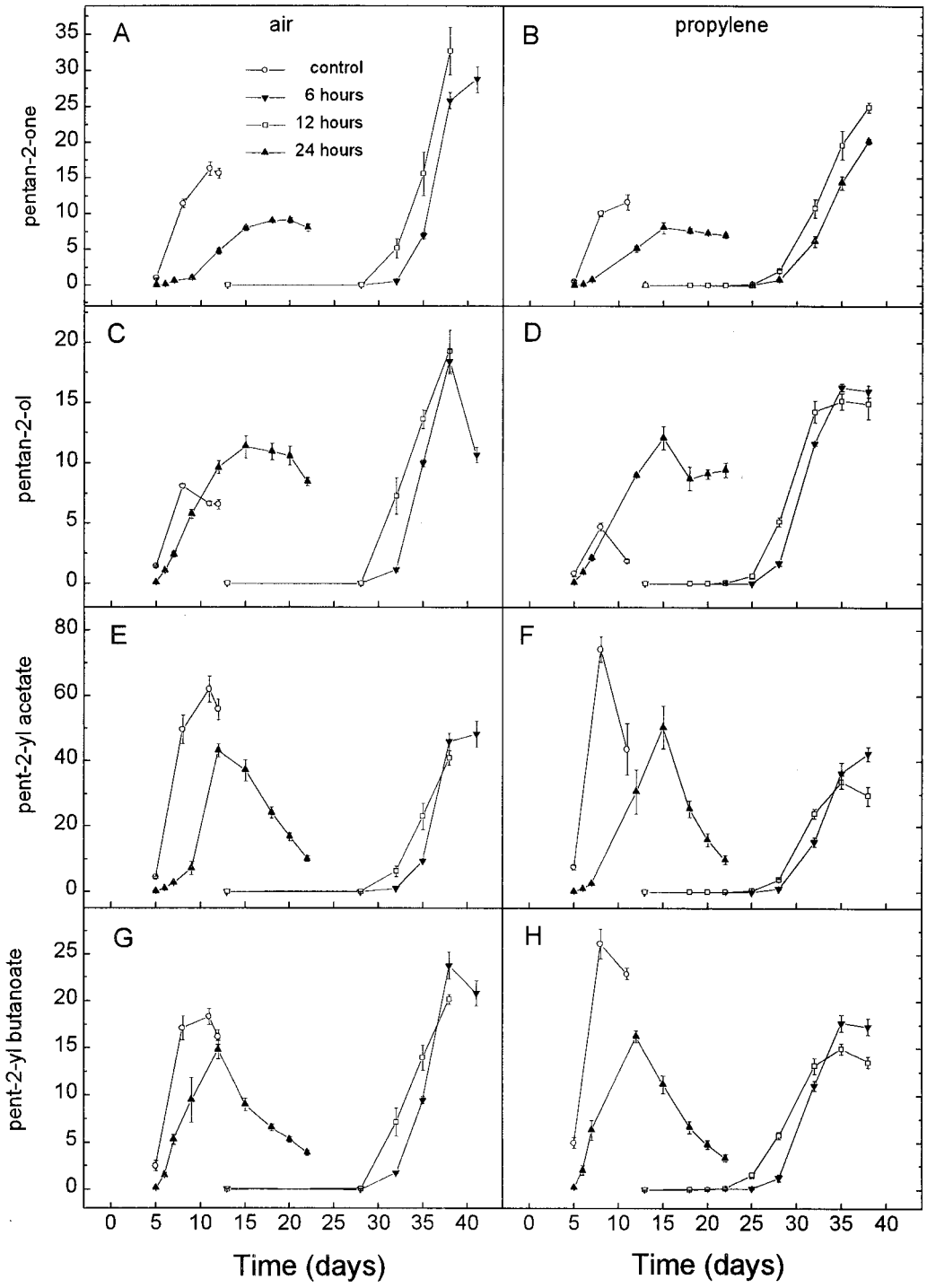

Figure 3. Rates of production (μg L^{-1} kg^{-1}) of pentan-2-one, pentan-2-ol, pent-2-yl acetate, and pent-2-yl butanoate by fruit treated with 1-MCP at 6, 12, and 24 HAPT. The control fruit had no 1-MCP. Following 1-MCP treatment, the fruits were either ventilated with humidified air (A, C, E, G) or treated continuously with propylene (B, D, F, H). The bars show the SEs of the means ($n = 3$). When absent, the SE bars fall within the dimensions of the symbol.

In contrast, 1-MCP treatment at 24 HAPT had only a small effect on the course of climacteric respiration and ethylene production compared to controls in both air and propylene environments. This suggests that many of the ripening processes were well under way when ethylene sensitivity was terminated at 24 HAPT, and those processes requiring only an ethylene trigger or which are developmentally controlled would continue much as for normal ripening. However, processes that are dependent on the continuing presence of ethylene would be inhibited.

All of the 1-MCP treatments used resulted in an increase in the rate of ethylene production and a decrease in respiration rates during ripening. Similarly, all 1-MCP treatments decreased the production of the total aroma volatiles. The most marked reduction (53%) was displayed by fruit treated 24 HAPT. This particular observation could be explained if the processes providing either enzymes, enzyme activation, or substrates critical to volatile production were dependent on the continuing presence of ethylene after the fruit was 24 h into the ripening sequence. The 6 and 12 HAPT 1-MCP-treated fruit also showed increased ethylene production, decreased respiration rate, and diminished (24−36%) total

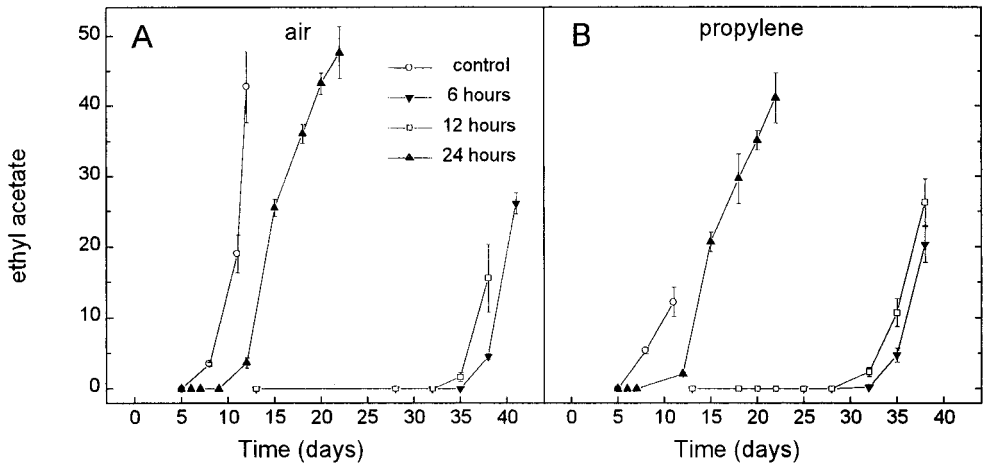

Figure 4. Rates of production (μg L^{-1} kg^{-1}) of ethyl acetate by fruit treated with 1-MCP at 6, 12, and 24 HAPT. The control fruit had no 1-MCP. Following 1-MCP treatment, the fruits were either ventilated with humidified air (A) or treated continuously with propylene (B). The bars show the SEs of the means (n = 3). When absent, the SE bars fall within the dimensions of the symbol.

volatile production. Because there is ample ethylene (and propylene) available to these fruit, the decrease in respiration and volatile production indicates either that a required ethylene-independent process has been completed before ripening or that ethylene sensitivity has not returned to the levels found in control fruit.

Changes in Volatile Composition. The changes in the development and composition of a suite of 12 compounds considered to be important components of banana aroma (Shiota, 1993) and constituting 74−90% of the total volatiles profile are shown in Figures 1−4. It is important to note that all of the experiments were stopped when the fruits were at the same apparent physiological age, that is, when subjectively judged fully ripe at peel color stage 7 (CSIRO, 1971). The continuous presence of the ethylene analogue (propylene) in the ripening environment had a small effect in hastening volatile production in 1-MCP-treated fruit but did not greatly affect total volatile production.

In general, the decrease in volatile production can be attributed to the large decline in the production of the esters, which constitute the majority of the normal profile. Exceptions to this trend are the esters 3-methylbutyl isovalerate and pent-2-yl butanoate, which both increased (in the range 11−60%) in 6 and 12 HAPT compared to control fruit. On the other hand, the branched-chain alcohols 2-methylpropanol and 3-methylbutanol and the straight-chain alcohol pentan-2-ol showed concentration increases of between 150 and 290%. It is noteworthy that despite these increases, the total concentrations of compounds containing the 3-methylbutyl, 2-methylpropyl, and butyl carbon skeletons are significantly below control values (27−54%), whereas those containing the 2-substituted pentyl skeleton are much less affected (0−29%).

It is apparent that the 1-MCP treatments have resulted in changes which influence the metabolic pathways leading to volatile formation. These changes have significantly altered the quantitative but not the qualitative composition of the aroma profile. The decrease in volatile formation could be attributed to a decrease in the concentrations or activity in the enzymes involved in their biosynthesis or to a lack of substrate availability. Bauchot et al. (1998) have shown that transgenic melons which produce little ethylene exhibit substantial decreases in volatile production during ripening and that this decline is more marked for those volatiles arising via amino acid metabolism. The structural units found in the carbon skeletons of banana esters are either straight-chain units thought to be derived from fatty acid metabolism or branched-chain units derived from amino acid catabolism (e.g., 3-methylbutyl and 2-methylpropyl derivatives) (Tressl and Drawert, 1973; Schreier, 1984).

The large increase in the concentrations of alcohols and the concomitant decrease in that of the esters in the treated fruit suggest that the final step in ester formation, the esterification of the alcohol and the acyl CoA catalyzed by the enzyme alcohol acyl transferase (AAT), is being disrupted. This could arise either from a decrease in the concentration or activity of AAT or by nonavailability of the necessary substrates. However, the relative increase in 3-methylbutyl isovalerate and pent-2-yl butanoate in the 6 and 12 HAPT bananas suggests that at least some AAT activity is maintained and that substrate availability, especially that of acyl CoA's from fatty acid metabolism, may be an important factor limiting ester formation. If this is the case, the accumulation of the alcohols suggests that ethylene has a differential affect on the metabolic pathways involved in volatile production, with the amino acid pathway being somewhat less affected than the fatty acid pathways which supply the even-carbon number straight skeletons. This conclusion is supported by the work of Song et al. (1997), who postulated that precursor supply was the factor that limits ester production in apples treated with 1-MCP. In addition, Harada et al. (1985) showed that although banana AAT activity was dependent on ethylene treatment, the onset of the activity preceded substrate availability. These results imply that some AAT activity may be present before the necessary substrates are present. Yoshioka et al. (1982) have shown that the enzymes involved in the ester biosynthetic sequence in bananas (AAT, α-keto decarboxylase, and ADH) show increased activity during ripening. They also showed similar substantial increases in the concentrations of potential substrates, especially the amino acid leucine, 3-methylbutanol, and acetic acid during ripening. These increases were found to precede the increase of key enzyme activity (Yoshioka et al., 1982). Hence, it appears likely that the processes responsible for both increasing substrate supply and the activation

of the ester biosynthesis system are ethylene dependent and that the lower volatile yield of 1-MCP-treated fruit reflects the tissue's reduced ethylene sensitivity. The decreased respiration rates of all treated fruit indicates a general decline in metabolic activity and hence the possibility of reduced substrate production. Song and Bangerth (1996) concluded that a general nonspecific increase in metabolic activity was a prerequisite for the stimulation of aroma production in apples because both straight- and branched-chain esters behaved similarly under the conditions of their experiments. A combination of all or some of these effects would account for the observed reduction in volatile production in 1-MCP-treated bananas.

Conclusion. The results support the postulate that ethylene plays an integrating role in many of the biochemical events that occur during the ripening of bananas. Inhibition of ethylene sensitivity with 1-MCP during the early stages of the climacteric produces changes in subsequent ripening behavior which reflect the disruption of this role.

The relative increase in alcohols and a decrease in their related esters indicate that ester composition may be determined by the supply of substrates and the activity of the ester biosynthesis enzymes, and the results suggest that the biochemical pathways controlling both of these factors are ethylene dependent.

ACKNOWLEDGMENT

We thank Dr. Mark Williams, Department of Chemistry, University of Western Sydney, Nepean, for the synthesis of 1-MCP. We also thank Tim Karlov for assisting in preliminary experiments.

LITERATURE CITED

Bauchot, A. D.; Mottram, D. S.; Dodson, A. T.; John, P. Effect of Aminocyclopropane-1-carboxylic Acid Oxidase Antisense Gene on the Formation of Volatile Esters in Cantaloupe Charentais Melon (Cv. Vedrandais). *J. Agric. Food Chem*. **1998**, *46*, 4787−4792.

Burg, S. P.; Burg, E. A. Molecular Requirements for the Biological Activity of Ethylene. *Plant Physiol*. **1967**, *42*, 144−152.

CSIRO (Commonwealth Scientific and Industrial Research Organisation). *Banana Ripening Guide*; Banana Research Advisory Committee; Technical Bulletin 3; Melbourne, Australia, 1971.

Golding, J. B.; Shearer, D.; Wyllie, S. G.; McGlasson, W. B. Application of 1-MCP and Propylene to Identify Ethylene Dependent Ripening Processes in Mature Banana Fruit. *Postharvest Biol. Technol*. **1998**, *14*, 87−98.

Harada, M.; Ueda, Y.; Iwata, T. Purification and Some Properties of Alcohol Acetyltransferase from Banana Fruit. *Plant Cell Physiol*. **1985**, *26*, 1067−1074.

Lelievre, J.-M.; Latche, A.; Jones, B.; Bouzayen, M.; Pech, J. C. Ethylene and Fruit Ripening. *Physiol. Plant*. **1997**, *101*, 727−739.

Magid, R. M.; Clarke, T. C.; Duncan, C. D. An Efficient and Convenient Synthesis of 1-Methylcyclopropene. *J. Org. Chem*. **1971**, *36*, 1320−1321.

McMurchie, E. J.; McGlasson, W. B.; Eaks, I. L. Treatment of Fruit with Propylene Gives Information About the Biogenesis of Ethylene. *Nature* **1972**, *237*, 235−236.

Sanz, C.; Olias, J. M.; Perez, A. G. Aroma Biochemistry of Fruits and Vegetables. In *Phytochemistry of Fruit and Vegetables*; Tomas-Barberan, F. A., Robins, R. J., Eds.; Clarendon Press: Oxford, U.K., 1997.

Schreier, P. *Chromatographic Studies of Biosynthesis of Plant Volatiles*; Dr. Alfred Hüthig Verlag: Heidelberg, 1984.

Shiota, H. New Esteric Compounds in the Volatiles of Banana Fruit (*Musa sapientum* L.). *J. Agric. Food Chem*. **1993**, *41*, 2056−2062.

Song, J.; Bangerth, F. The Effect of Harvest Date on Aroma Compound Production from 'Golden Delicious' Apple Fruit and Relationship to Respiration and Ethylene Production. *Postharvest Biol. Technol*. **1996**, *8*, 259−269.

Song, J.; Tian, M. S.; Dilley, D. R.; Beaudry, R. M. Effect of 1-MCP on Apple Fruit Ripening and Volatile Production. *HortScience* **1997**, *32*, 536.

Tressl, R.; Drawert, F. Biogenesis of Banana Volatiles. *J. Agric. Food Chem*. **1973**, *21*, 560−565.

Tressl, R.; Jennings, W. G. Production of Volatile Compounds in the Ripening Banana. *J. Agric. Food Chem*. **1972**, *20*, 189−192.

Wyllie, S. G.; Golding, J. B.; McGlasson, W. B.; Williams, M. The Relationship Between Ethylene and Aroma Volatiles Production in Ripening Climacteric Fruit. In *Food Flavours: Formation, Analysis and Packaging Influences*; Contis, E. T., Ho, C.-T., Mussinan, C. J., Parliment, T. H., Shahidi, F., Spanier, A. M., Eds.; Developments in Food Science 40; Elsevier: Amsterdam, 1998; pp 375−384.

Yoshioka, H.; Ueda, Y.; Iwata, T. Development of Isoamyl Acetate Biosynthetic Pathway in Banana Fruit during Ripening and Suppression in Its Development at High Temperature. Physiological Studies of Fruit Ripening in Relation to Heat Injury. Part 4. *Nippon Shokuhin Kogyo Gakkaishi* **1982**, *29*, 333−339.

Received for review August 13, 1998. Revised manuscript received January 20, 1999. Accepted January 25, 1999.

JF980906C