10. Heinicke, A. J. 1922. Some relations between the circumference and weight, and between root and top growth of young apple trees. *Proc. Amer. Soc. Hort. Sci.* 18:222-227.
11. Hoblyn, T. N. 1931. Field experiments in horticulture. *Imp. Bur. Fruit Prod., Tech. Comm.* 2:1-50.
12. Holland, D. A. 1959. Studies in the measurement of apple trees. IV. The use of branch girths to estimate tree size. Annu. Rpt. E. Malling Res. Sta. for 1958. p. 77-79.
13. Pearce, S. C. 1949. The variability of apple trees. I. The extent of crop variation and its minimization by statistical means. *J. Hort. Sci.* 25:3-9.
14. _____. 1952. Studies in the measurement of apple trees. I. The use of trunk girths to estimate tree size. Annu. Rpt. E. Malling Res. Sta. for 1951. p. 101-104.
15. _____ 1956. Studies in the measurement of apple trees. III. The use of branch girths as a substitute for the trunk girth. Annu. Rpt. E. Malling Res. Sta. for 1955. p. 76-79.
16. Preston, A. P. 1954. Apple rootstocks studies: the M. IX crosses. Annu. Rpt. E. Malling Res. Sta. for 1953. p. 89-94.
17. _____. 1958. Apple rootstock studies: Thirty-five years' results with Lane's Prince Albert on clonal rootstocks. *J. Hort. Sci.* 33:29-38.
18. _____. 1967. Apple rootstock studies: Fifteen years' results with some M. IX crosses. *J. Hort. Sci.* 42:41-50.
19. _____, and J. Ingram. 1962. Apple rootstock studies: The behavior of twelve clonal rootstocks at Luddington. *Expt. Hort.* 6:84-90.
20. Sudds, R. H. and R. D. Anthony. 1928. The correlation of trunk measurements with tree performance in apples. *Proc. Amer. Soc. Hort. Sci.* 25:244-246.
21. Taylor, R. B. 1966. A study of correlations relating branch and trunk circumference to yield of apple trees. MSc Thesis, Univ. of Mass. Amherst.
22. Tingley, M. A. and G. F. Potter. 1934. Effect of crop and treatment on winter injury to Baldwins. *Proc. Amer. Soc. Hort. Sci.* 32:234-238.
23. Tukey, H. B. and K. D. Brase. 1938. Studies of top and root growth of young apple trees in soil and peat-soil mixtures of varying moisture contents. *Proc. Amer. Soc. Hort. Sci.* 36:18-27.
24. Waring, J. H. 1920. The probable value of trunk circumference as an adjunct to fruit yield in interpreting apple orchard experiments. *Proc. Amer. Soc. Hort. Sci.* 17:179-185.
25. Westwood, M. N. and A. N. Roberts. 1970. The relationship between trunk cross-sectional area and weight of apple trees. *J. Amer. Soc. Hort. Sci.* 95:28-30.
26. Wilcox, J. C. 1937. Field studies of apple tree growth and fruiting. III. Some observations on the measurement of tree vigour. *Sci. Agr.* 17:657-669.
27. _____. 1940. Adjusting apple yield for differences in size of tree. *Sci. Agr.* 21:139-148.

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Phytotoxicity of Ethylene Dibromide to Cherry and Banana Fruit[1]

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Abstract. **Fruit of cherry (*Prunus avium* L.) and banana (*Musa* sp.,) AAA Group, Cavendish sub-group cv. Williams Hybrid, when fumigated with ethylene dibromide (EDB) and held at 20°C were injured visibly by treatment concentrations of 32 g/m^3 or more. EDB (4 g/m^3) stimulated the rates of both ethylene (C_2H_4) evolution and respiration in cherries, while higher concentrations up to 32 g/m^3 caused proportionately greater increases in the rates of gas exchange. Cherries stored at 1° after fumigation with 32 g/m^3 EDB did not display the increases in gas exchange which were observed at 20°, but during a 7 day storage period severe symptoms of phytotoxicity developed. The increases in gas exchange are, therefore, effects and not causes of EDB injury. The stimulation of C_2H_4 production in cherries by EDB was reduced by pretreatment with Co^{2+}, indicating that EDB affects the methionine pathway of ethylene synthesis. In bananas treated with 4 g/m^3 EDB and held at 20°, the respiration rate increased but C_2H_4 evolution and electrolyte leakage from slices of pulp tissue were unaffected. When the EDB concentration was raised to 32 g/m^3, respiration and C_2H_4 evolution rates and electrolyte leakage increased.**

Fumigation with EDB is used widely as a quarantine treatment of fruit against several species of fruit fly. A serious problem encountered with EDB fumigation is that concentrations of the compound which have efficient insecticidal activity may also be phytotoxic. As part of a program to develop a fumigation schedule for sweet cherries against *Dacus tryoni* Frogg., a fruit fly indigenous to north-eastern Australia (5), we have studied the effects of a range of EDB concentrations on respiration rate and C_2H_4 production of sweet cherries stored at 1° and 20°C. We have compared the responses to fumigation of the non-climacteric cherry (3) with those of the climacteric banana (2) and examined an earlier suggestion (16) that EDB may alter the permeability of plant tissues. The ability of cobalt ions to inhibit conversion of methionine to ethylene (11) was used to furnish presumptive evidence that the methionine pathway of ethylene biogenesis is affected by EDB treatment.

Materials and Methods

'Early Lyons' and 'Eagle Seedling' cherries were harvested at Young, N.S.W., and dipped in fungicide (500 mg/liter

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benomyl and 750 mg/liter dichloran). Fruit were fumigated with EDB for 2 hr at 20°C as described previously (15, 16). Some fruit were pretreated before fumigation by immersion for 2 min at 20°C in solutions of 0.10 M $CoCl_2$ or 0.15 M KCl containing surfactant (0.005% by volume nonyl phenol ethylene oxide condensate). After fumigation and ventilation in air at 20°, fruit were placed in jars ventilated with moist air at either 1 or 20° (±0.5°). Rates of respiration and C_2H_4 evolution were determined by analysis of the effluent airstreams (12, 14).

Bananas were harvested at Alstonville, N.S.W., fumigated with EDB and placed in respiration jars at 20°C as above. Transverse 2 mm-thick slices of pulp tissue were cut into 4 equal sectors, and 14 sectors (about 5 g) bathed in 50 ml of aerated deionized water and washed for 1 hr at 0°. Electrolyte leakage was estimated by measuring the conductivity of the bathing solution before and after boiling the tissue/water suspension for 10 min. Both measurements were made with the tissue in contact with the bathing solution at 0°.

Results

Effects of fumigation on gas exchange of cherries at 20°C. The postharvest respiration rate of untreated cherries declined gradually (Fig. 1). Fumigation with EDB abolished this respiratory decline and particularly in 'Early Lyons' caused a gradual respiratory rise. Respiratory rates and total CO_2 evolved increased relative to controls as the concentration of EDB increased from 0 to 32 g/m³ (Fig. 1, 2). The highest concentration of EDB tested (44 g/m³) caused no further increase in respiration rates and actually depressed the rates slightly relative to the 32 g/m³ treatment. Treatment with EDB had no effect whatever on the respiratory quotient, which was 1.1 to 1.2 in all treatments.

Fumigation with EDB substantially increased the rates of C_2H_4 evolution in both cultivars (Fig. 1). A peak in C_2H_4

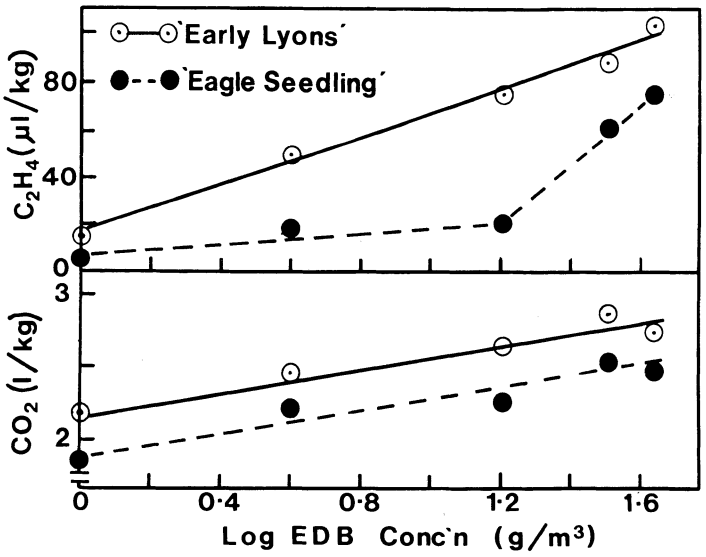

Fig. 2. Dose-response curves showing effects of EDB concentration on total CO_2 and C_2H_4 evolved by 'Early Lyons' and 'Eagle Seedling' cherry fruit. Estimated by integration of each curve in Fig. 1.

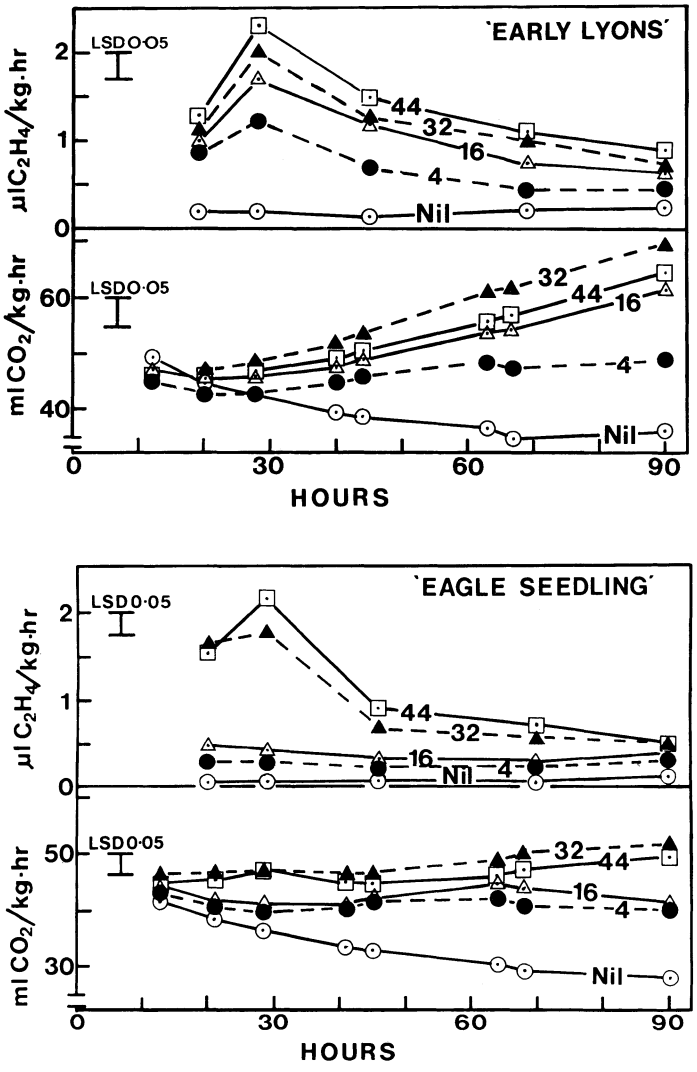

Fig. 1. Respiration and C_2H_4 evolution rates of 'Early Lyons' and 'Eagle Seedling' cherry fruit fumigated with 0 or 4, 16, 32 and 44 g/m³ EDB at time zero. Each result is derived from 3 replicates, each of 100 g fruit.

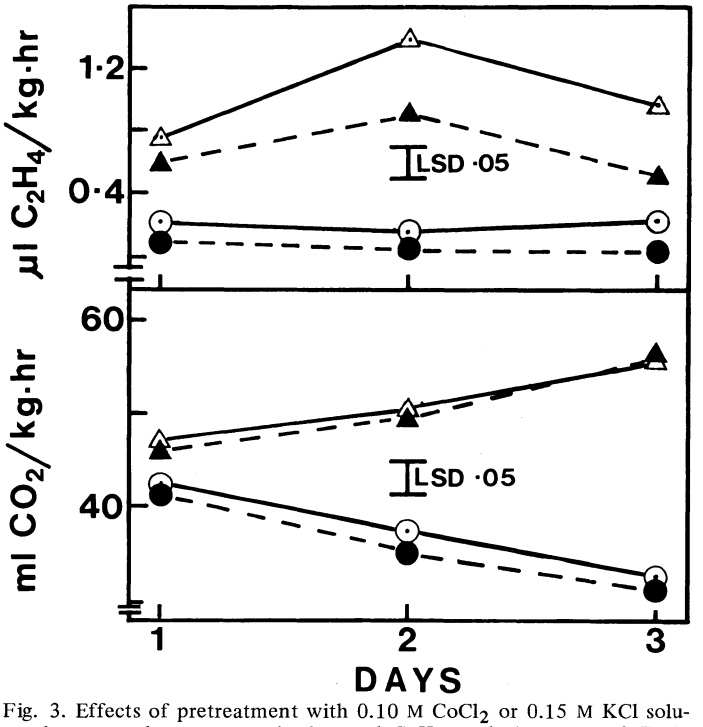

Fig. 3. Effects of pretreatment with 0.10 M $CoCl_2$ or 0.15 M KCl solutions on subsequent respiration and C_2H_4 evolution rates of 'Early Lyons' cherry fruit fumigated with 0 or 32 g/m³ EDB at zero time. Treatments: $CoCl_2$, 0 EDB ● – – – ●; $CoCl_2$, 32 g/m³ EDB ▲ – – – ▲; KCl, 0 EDB ○ —— ○; KCl, 32 g/m³ EDB △ —— △. Each result is derived from 3 replicates, each of 100 g fruit.

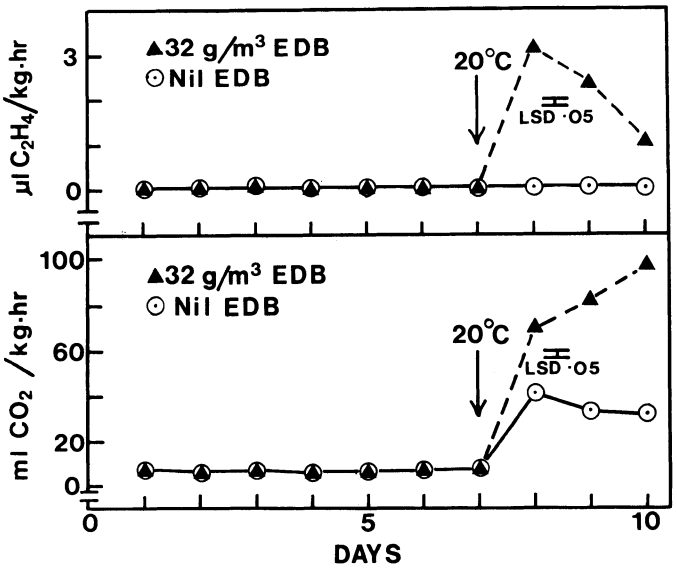

Fig. 4. Respiration and C_2H_4 evolution rates of 'Eagle Seedling' cherry fruit fumigated with 0 or 32 g/m^3 EDB at zero time and stored at 1°C for 7 days. On the 7th day samples of fruit were transferred to 20°. There was no change in the rates of gas exchange by fruit left at 1° (results not shown). Each result is derived from 3 replicates at 1° each of 700 g fruit, or 4 replicates at 20°, each of 100 g fruit.

evolution occurred within 40 hr of treatment. The rates of C_2H_4 evolution and total C_2H_4 evolved increased as EDB concentration increased above a minimum threshold level (Fig. 1, 2). This threshold was higher for 'Eagle Seedling' than for 'Early Lyons', due to a cultivar or stage of maturity difference in either uptake of EDB or sensitivity to the compound. In contrast to respiration rate, 44 g/m^3 EDB caused a further increase (not a depression) in C_2H_4 evolution, relative to the 32 g/m^3 treatment.

Nature of the increase in C_2H_4 evolution. When cherries which had been briefly immersed in Co^{2+} or K^+ solutions were fumigated with 32 g/m^3 EDB, stimulation of C_2H_4 production was reduced significantly in the fruit pretreated with Co^{2+} (Fig. 3). Penetration of Co^{2+} was probably incomplete. The control treatment was immersion in a K^+ solution which was equiosmolar with the Co^{2+} solution. Making the solutions equimolar with respect to either cation or anion concentration gave the same result. Respiration rate was unaffected by Co^{2+} pretreatment (Fig. 3).

Effects of EDB fumigation on gas exchange of cherries stored at 1°C and later transferred to 20°. No effects of EDB fumigation (32 g/m^3) on gas exchange were discerned 24 hr after fruit were placed in storage at 1° (Fig. 4). Both cultivars showed similar behavior, and results for 'Eagle Seedling' only are shown. When, however, fruit were transferred from 1° to 20° after 7 days storage, a response to prior fumigation was observed. A burst of C_2H_4 evolution accompanied the transfer to 20° in fumigated fruit only. This was accompanied by a large and sustained increase in respiration rate.

Visible phytotoxic effects of EDB fumigation of cherries. Severe injuries developed in fruit of both cultivars treated with 44 g/m^3 EDB and held at 20°C for 7 days. Symptoms were spreading necrotic lesions in the flesh and exudation of juice. Similar, but less severe injury occurred in 'Early Lyons' fruit at 20° treated with 32 g/m^3 EDB. Severe injury was evident in fruit of both cultivars treated with 32 g/m^3 EDB and held at 1° for 7 days. Upon transfer to 20° the necrotic lesions continued to enlarge rapidly.

In this and other trials, fumigation with as little as 4 g/m^3 EDB appeared to increase the incidence of fungal diseases. Brown rot incited by *Sclerotinia fructicola* (Wint.) Rehm was found in EDB-treated fruit at 20°C, whereas this disease was almost absent in unfumigated controls (all fruit were treated with benomyl to control brown rot). Diseases which are usually found in cherries after several weeks of cool-storage were quite common in EDB-treated fruit at 20°. Organisms found included species of *Penicillium, Alternaria* and *Botrytis.*

Effects of EDB fumigation on bananas. Bananas were treated with EDB to determine if their gas exchange, like that of cherries, was affected by concentrations of fumigant which did not cause visible injury. The pulp of unripe bananas was also tested for EDB-induced permeability changes because the leakage characteristics of this tissue are known (4). EDB may affect the permeability of cell membranes, and gross changes in membrane permeability can be detected by measuring the leakage of small molecules from tissue slices.

Fumigation of preclimacteric bananas with 4 g/m^3 EDB increased whole-fruit respiration rate, but had no significant effect on whole-fruit C_2H_4 evolution or electrolyte leakage from pulp slices (Table 1). These fruit suffered no visible injury. A concentration of 32 g/m^3 EDB caused increases in respiration rate, C_2H_4 evolution and leakage. A brown discoloration of the skin was observed in fruit from this treatment. Bananas did not soften or accumulate soluble solids when treated with 4 or 32 g/m^3 EDB. The intermediate concentrations of 7.5 and 15 g/m^3 can, however, initiate ripening in bananas (8).

Table 1. Effects of EDB fumigation on respiration rate, C_2H_4 evolution and electrolyte leakage of banana fruit.[z]

EDB concn (g/m^3)	Time after fumigation									
	4 hr	24 hr			48 hr			72 hr		
	Leakage[y] (%)	Respn[x] rate	C_2H_4[w] rate	Leakage (%)	Respn rate	C_2H_4 rate	Leakage (%)	Respn rate	C_2H_4 rate	Leakage (%)
0	25 cd[v]	13.0 a	–[u]	22 ab	13.1 a	–	23 bc	14.2 ab	–	24 bcd
4	27 e	22.0 c	–	22 ab	17.5 b	–	22 ab	16.6 ab	–	19 a
32	26 cd	33.1 d	0.22 c	26 cd	35.5 de	0.15 b	28 e	37.4 e	0.09 a	27 e

[z]Each result is the mean of assays on 4 individual fruit.

[y](Electrolyte which leaked out/total electrolyte) x 100%.

[x]ml CO_2/kg·hr.

[w]µl C_2H_4/kg·hr.

[v]Mean separation in all columns of each parameter by Duncan's multiple range test, 5% level.

[u]Below the limit of detection, which was ≤ 0.05 µl/kg·hr.

Discussion

The physiological effects of EDB upon fruits change both qualitatively and quantitatively as the concentration of fumigant increases. A relatively low concentration of EDB (4 g/m^3) stimulates respiration rate and C_2H_4 evolution in cherries and respiration rate in bananas (Fig. 1, Table 1). When applied to green tomatoes, 4 g/m^3 EDB hastened softening and enhanced lycopene accumulation (16). Ripening was initiated in bananas treated with 7.5 and 15 g/m^3 EDB (8). Respiration rate and softening were stimulated in avocados treated with 16 g/m^3 EDB (6). These effects of EDB fumigation are similar to those of relatively low concentrations of hydrogen cyanide, bromine and carbon monoxide. At concentrations of 400 – 1000 μl/liter (cf. 4 g/m^3 EDB, which is about 500 μl/liter), these compounds, which are believed to interfere with cellular organization, initiated ripening in bananas and avocados (17, 18).

The quantitative effects of EDB are shown by the dose-response plots of Fig. 2. Progressive increases in respiration rate as a function of EDB concentration (8 – 16 g/m^3) have also been shown in citrus fruit (7). The net effect of EDB interaction with a tissue may be a concentration-dependent stimulation of some metabolic processes and inhibition of others, so that at low concentrations EDB mimics C_2H_4 by increasing gas exchange rates and initiating ripening. At the relatively high concentrations required for insect disinfestation, inhibitory effects predominate. Ripening is prevented and tissue death occurs.

In common with C_2H_4 (4), EDB (4 g/m^3) stimulated respiration rate in bananas without affecting gross tissue leakage, although more subtle effects of EDB on membranes are not precluded by this result. An EDB treatment (32 g/m^3) which increased both respiration rate and C_2H_4 evolution in bananas did increase tissue leakage. This increased leakage may have arisen from cells killed by EDB, or from living cells rendered more permeable by the fumigant. Similarly, the decreased response of cherry respiration to 44 g/m^3 EDB may have been due to cell death, or to an inhibitory effect of this amount of EDB on respiration of live cells. The increases in gas exchange of cherries fumigated with EDB are characteristic of the stress response of wounded non-climacteric fruits (10). The increased C_2H_4 evolution probably arose from the methionine pathway, which appears to be the source of stress ethylene (1, 9).

Cherries stored at 1^{o}C after fumigation with 32 g/m^3 EDB suffered more severe injury than those stored at 20^o. Storage at 1^o abolished the increases in gas exchange observed in fumigated fruit at 20^o, which establishes that these increases are effects and not causes of EDB injury. Prompt cooling of EDB-fumigated fruit exacerbates phytotoxicity, and with commercial-size loads of fruit a 24 hr delay between fumigation and commencement of cooling may be required (13). This injurious effect of cooling might be ascribed to a higher residue of unchanged fumigant persisting in the cooled fruit, or to a decreased capacity of the fruit to repair cellular damage at low temperature.

Literature Cited

1. Abeles, A. L. and F. B. Abeles. 1972. Biochemical pathway of stress-induced ethylene. *Plant Physiol.* 50:496-498.
2. Biale, J. B. 1960. Respiration of fruits. *In* W. Ruhland (ed.) Encyclopedia of plant physiology 12 (2):536-592. Springer-Verlag, Berlin.
3. Blanpied, G. D. 1972. A study of ethylene in apple, red raspberry, and cherry. *Plant Physiol.* 49:627-630.
4. Brady, C. J., P. B. H. O'Connell, J. Smydzuk, and N. L. Wade. 1970. Permeability, sugar accumulation, and respiration rate in ripening banana fruits. *Austral. J. Biol. Sci.* 23:1143-1152.
5. Colless, D. H. and D. K. McAlpine. 1970. Diptera. *In* F. W. Waterhouse (ed.) The insects of Australia. Melbourne University Press, Melbourne.
6. Eaks, I. L. and W. B. Sinclair. 1955. Respiratory response of avocado fruits to fumigation effective against the eggs and larvae of fruit flies. *J. Econ. Entomol.* 48:369-372.
7. _____and W. A. Ludi. 1958. Response of orange and lemon fruits to fumigation with ethylene dibromide effective against eggs and larvae of the Oriental and Mexican fruit flies. *Proc. Amer. Soc. Hort. Sci.* 72:297-303.
8. Farooqi, W. A. and E. G. Hall. 1972. Effects of ethylene dibromide on the respiration and ripening of bananas (*Musa cavendishii* L.). *Nucleus* 9 (3-4):22-28.
9. Hanson, A. D. and H. Kende. 1976. Biosynthesis of wound ethylene in morning-glory flower tissue. *Plant Physiol.* 57:538-541.
10. Herner, R. C. and K. C. Sink, Jr. 1973. Ethylene production and respiratory behavior of the *rin* tomato mutant. *Plant Physiol.* 52: 38-42.
11. Lau, O.-L. and S. F. Yang. 1976. Inhibition of ethylene production by cobaltous ion. *Plant Physiol.* 58:114-117.
12. McGlasson, W. B. 1969. Ethylene production by slices of green banana fruit and potato tuber tissue during the development of induced respiration. *Austral. J. Biol. Sci.* 22:489-491.
13. Norman, G. G., W. Grierson, T. A. Wheaton, and J. D. Dennis. 1976. Minimizing hazards from in-truck ethylene dibromide fumigation of carton-packed citrus fruit. *Proc. Fla. State Hort. Soc.* 88:323-328.
14. Palmer, J. K. and W. B. McGlasson. 1969. Respiration and ripening of banana fruit slices. *Austral. J. Biol. Sci.* 22:87-99.
15. Rigney, C. J. and B. L. Wild. 1975. *Dacus tryoni:* ethylene dibromide treatment of oranges. *J. Econ. Entomol.* 68:653-654.
16. _____, D. Graham, and T. H. Lee. 1978. Changes in tomato fruit ripening caused by ethylene dibromide fumigation. *J. Amer. Soc. Hort. Sci.* 103:420-423.
17. Solomos, T. and G. G. Laties. 1973. Cellular organization and fruit ripening. *Nature* 245:390-392.
18. _____ and _____. 1974. Similarities between the actions of ethylene and cyanide in initiating the climacteric and ripening of avocados. *Plant Physiol.* 54:506-511.