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The Role of Ethylene in Seed Dormancy with Particular Reference to Apple (*Malus domestica* Bork H.) Seeds

WAN CHEE KEONG
Department of Agronomy and Horticulture, Faculty of Agriculture, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia

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RINGKASAN

Peranan ethylene dalam pencegahan rehat dan percambahan biji-benih epal telah dikajikan. Perkaitan kadar ethylene berasal dari dalam bijibenih dengan kebolehan percambahannya tidak diperolehi. Penambahan ethephon (2-chloroethylphosphonic asid) ke dalam "stratification medium" menggalakkan pengeluaran ethylene oleh bijibenih, tetapi tidak ada kesan yang tegas terhadap percambahannya. Bahan-bahan kimia yang menahan pergerakan atau sintesis ethylene, seperti 8-hydroxyquinoline sulfat dan silver nitrat, mengurangi pengeluaran ethylene, tetapi tidak menahan percambahan bijibenih pada aras yang bukan tosik. Keputusannya bijibenih epal tidak memerlukan ethylene untuk mengatasi rehatnya. Selain dari itu percambahannya bukan sekejadian disebabkan oleh ethylene.

SUMMARY

The involvement of ethylene in the regulation of apple seed dormancy and germination was investigated. No relationship could be established between the rate of endogenous ethylene biosynthesis by seeds and their ability to germinate. Addition of ethephon (2-chloroethylphosphonic acid) to the stratification medium promoted ethylene production by seeds, but had no consistent effect on germination. The presumed inhibitor of ethylene action or synthesis, 8-hydroxyquinoline sulfate and silver nitrate, reduced ethylene production but did not inhibit germination at non-toxic levels. It is concluded that ethylene is not essential for breaking dormancy in apple seeds, nor is germination an ethylene-induced response.

INTRODUCTION

The phenomenon of seed dormancy is under hormonal control (Amen, 1968; Frankland, 1961; Koller *et al.*, 1962; Taylorson and Hendricks, 1977; and Overbeek, 1966). Ethylene and ethrel, an ethylene releasing compound, have been reported to be capable of releasing dormancy and stimulating, germination in various seeds (Brenan *et al.*, 1978; Globerson, 1977; Kepczynski *et al.*, 1977). Recently, it has been suggested that ethylene is one of the hormones regulating dormancy in apple seeds (Kepczynski *et al.*, 1975, 1976 and 1977). Kepczynski *et al.* (1977) observed that the production of ethylene by excised embryos paralleled their germination capacity during after-ripening. However, exogenous application of ethephon was not effective in accelerating the germination of partially-stratified seeds (Halinska *et al.*, 1975; and Sinha *et al.*, 1977). This raises doubts as to the involvement of ethylene in the regulation of dormancy in apple seeds. The objective of this study was to establish the extent of ethylene requirement in dormancy in intact apple seeds.

MATERIALS AND METHODS

Seeds were removed from apple fruits, cv 'Paulared', which had been stored at 5°C ± 1°C for 16 weeks. Secondary dormancy was induced by holding the seeds at 32°C ± 2°C for three weeks under moist conditions. At the end of this period the embryos were excised and test germinated to ensure they were in a state of dormancy. Seeds were then soaked for 24 hours in: (a) distilled water (control), (b) ethephon, (c) 8-hydroxyquinoline sulfate (8-HQS), and (d) silver nitrate ($AgNO_3$), and subsequently

Key to author's name: Wan, C.K.

stratified in petri-dishes lined with filter paper moistened with the appropriate chemical test solutions. The experiment was repeated with fresh (primary dormant) seeds of the same cultivar.

Ethylene production was measured by placing 30 seeds in a 25 ml flask lined with filter paper moistened with distilled water. The flasks were closed with rubber serum caps and incubated in the dark at 20°C ± 1°C for 24 hours. Five gas samples were drawn from each flask with a 1 cc syringe and the ethylene content was determined by using a Varian Aerograph series 1700 gas chromatograph equipped with a flame ionization detector and a column (45 × 0.32 cm) of 60 to 80 mesh aluminium oxide operated at 60° or 80°C. One cc of ambient air was put back into the flask after each time a sample of gas was drawn. Gas samples were also taken from a flask lined with filter paper moistened with distilled water without seeds and used to correct for ambient levels of ethylene. All values were converted to $nl.g^{-1}$ of seed hr^{-1}. Two replications were used for each treatment.

Germination tests were carried out with three replications of 20 seeds in 9-cm petri-dishes lined with two layers of whatman no. 1 filter paper moistened with distilled water. The petri-dishes were randomly placed in a growth chamber at 20°C ± 1°C in darkness. Seeds with visible radicle protrusion were considered to be germinated. Germination was recorded for a period of ten days, and the results expressed as mean percentages.

The data from the study were subjected to analysis of variance and the mean separation procedure of Duncan's Multiple Range Test was used.

RESULTS AND DISCUSSION

Dormant, both secondary and primary, apple seeds were found to produce endogenous ethylene which declined with time of stratification (Table 1). The ethylene production was lower in primary dormant seeds, and the decline was rapid rather than gradual. Pre-soaking and stratifying seeds in ethephon in various concentrations from 100 to 1000 ppm significantly stimulated ethylene evolution by the seeds. Treatment with ethephon at 100 ppm was more promotive in stimulating ethylene production than the higher concentration, possibly due to supra-optimal concentration.

The effect of ethephon treatment on germination are shown in Table 2. Germination was not increased nor enhanced by ethephon treatment. In fact, ethephon inhibited germination of primary dormant seeds at 250 or 500 ppm after six and/or nine weeks, the higher concentration being less effective than the lower. This is consistent with the observations of others (Halinska *et al.*, 1975, and Sinha *et al.*, 1977) showing that ethephon is not effective in stimulating the germination of partially stratified seeds. Data obtained show that ethylene does not have any effect on breaking dormancy. Also, no consistent relationship between ethylene evolution and germination could be established in either secondary or primary dormant seeds. If ethylene is required to break the dormancy of seeds, the addition to the stratification medium of chemical compounds which inhibit ethylene biosynthesis or block ethylene action should affect germination. Treatment with 8-HQS effectively reduced the ethylene biosynthesis in dormant seeds as have been shown for other

TABLE 1

The effects of ethephon treatment during moist stratification on the ethylene production of apple, cv. 'Paulared', seeds.

Treatment	Concn (ppm)	C_2H_4 production ($nl.g^{-1}.hr^{-1}$) after stratification (wk)*								
		4	6	9	12	0	4	6	9	12
		Secondary dormant				Primary dormant				
Water	–	2.84b	1.38c	0.66c	0.05c	3.42	0.07b	0.17b	0.07c	1.26c
Ethephon	100	33.29a	43.12a	47.58a	24.88a	–	–	–	–	–
	250	–	–	–	–	–	3.97a	2.95a	14.60b	15.28a
	500	–	–	–	–	–	4.66a	3.75a	28.14a	7.14b
	1000	6.86b	18.78b	39.93b	12.01b	–	–	–	–	–

*Means within columns followed by the same letter are not significantly different at the 5% elvel (DMRT).

tissues (Parups and Paterson, 1975). Silver ion, which was shown to strongly block ethylene action (Beyer, 1976), proved to be as effective as 8-HQS in inhibiting the formation of ethylene (Table 3). The higher concentration (200 ppm) of 8-HQS inhibited seed germination after four, six and nine weeks of stratification but not thereafter (Table 4). $AgNO_3$ at 50 or 100 ppm also inhibited germination at six weeks, but not at nine or twelve weeks.

TABLE 2
The effects of treatment with ethephon during moist stratification on germination of apple, cv. 'Paulared', seeds.

Treatment	Concn (ppm)	Germination (%) wks after stratification*							
		4	6	9	12	4	6	9	12
		Secondary dormant				Primary dormant			
Water	–	15a	47a	93a	100a	5a	20a	87a	98a
Ethephon	100	13a	42a	90a	100a	–	–	–	–
	250	–	–	–	–	0a	0b	17b	100a
	500	–	–	–	–	0a	7b	88a	100a
	1000	18a	53a	97a	100a	–	–	–	–

*Means within columns followed by the same letter are not significantly different at the 5% level (DMRT).

TABLE 3
The effects of treatment with 8-hydroxyquinolinge sulfate and silver nitrate during moist stratification on ethylene production of apple, cv. 'Paulared', seeds.

Treatment	Concn (ppm)	C_2H_4 production ($nl.g^{-1}.hr^{-1}$.) after stratification (wk)*							
		4	6	9	12	4	6	9	12
		Secondary dormant				Primary dormant			
Water	–	2.84a	1.38a	0.66a	0.06a	0.07a	0.17a	0.07a	1.26a
8-HQS	100	0.32b	0.28b	0.24b	0.04a	0.02b	0.09b	0.02b	0.09b
	200	0.20b	0.20b	0.10c	0.04a	0.02b	0.09b	0.02b	0.06b
$AgNO_3$	50	0.02c	0.18b	0.12c	0.04a	0.04b	0.09b	0.04b	0.08b
	100	0.02c	0.08b	0.10c	0.04a	0.04b	0.09b	0.03b	0.08b

*Means within columns followed by the same letter are not significantly different at the 5% level (DMRT).

TABLE 4
The effects of treatment with 8-hydroxyquinoline sulfate and silver nitrate during moist stratification on germination of apple, cv. 'Paulared' seeds.

Treatment	Concn (ppm)	Germination (%) wks after stratification*							
		4	6	9	12	4	6	9	12
		Secondary dormant				Primary dormant			
Water	–	15ab	47ab	93a	100a	5a	20a	87ab	98a
8-HQS	100	17ab	37ab	85b	100a	3ab	18a	93a	98a
	200	5c	23b	58c	98a	2ab	10ab	67b	98a
$AgNO_3$	50	13b	48a	98a	100a	0b	5b	83ab	100a
	100	22a	45ab	98a	100a	2ab	3b	78ab	100a

*Means within columns followed by the same letter are not significantly different at the 5% level (DMRT).

The above data taken together, do not support the hypothesis that ethylene participates in the regulation of dormancy and germination of apple seeds.

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