Plant Physiol. (1972) 50, 765–768

Ethylene: Response of Fruit Dehiscence to CO_2 and Reduced Pressure[1]

Received for publication May 23, 1972

JOHN A. LIPE[2] AND PAGE W. MORGAN
Department of Plant Sciences, Texas A&M University, College Station, Texas 77843

ABSTRACT

These studies were conducted to determine whether ethylene serves as a natural regulator of fruit wall dehiscence, a major visible feature of ripening in some fruits. We employed treatments to inhibit ethylene action or remove ethylene and observed their effect on fruit dehiscence. CO_2 (13%), a competitive inhibitor of ethylene action in many systems, readily delayed dehiscence of detached fruits of cotton (*Gossypium hirsutum* L.), pecan (*Carya illinoensis* [Wang.] K. Koch), and okra (*Hibiscus esculentus* L.). The CO_2 effect was duplicated by placing fruits under reduced pressure (200 millimeters mercury), to promote the escape of ethylene from the tissue. Dehiscence of detached fruits of these species as well as *attached* cotton fruits was delayed. The delay of dehiscence of cotton and okra by both treatments was achieved with fruit harvested at intervals from shortly after anthesis until shortly before natural dehiscence. Pecan fruits would not dehisce until approximately 1 month before natural dehiscence, and during that time, CO_2 and reduced pressure delayed dehiscence. CO_2 and ethylene were competitive in their effects on cotton fruit dehiscence. All of the results are compatible with a hypothetical role of ethylene as a natural regulator of dehiscence, a dominant aspect of ripening of cotton, pecan, and some other fruits.

Evidence that ethylene is a natural regulator of fruit dehiscence prompted this investigation of the ability of CO_2 and reduced pressure to regulate fruit dehiscence (9). CO_2 interferes with responses of plant tissues to ethylene (8); leaf abscission is one response so affected (1). More recently, storage of fruits at reduced atmospheric pressure was shown to delay fruit ripening, apparently by hastening escape of endogenous ethylene (3–5). If ethylene produced by the fruit causes dehiscence, then fumigation with CO_2 or storage at reduced pressure should delay dehiscence and both effects are reported here.

MATERIALS AND METHODS

Fumigation with CO_2. Detached fruits of cotton (*Gossypium hirsutum* L.), pecan (*Carya illinoensis* [Wang.] K. Koch), and okra (*Hibiscus esculentus* L.) were fumigated with CO_2 and the effects on dehiscence were observed. Cotton fruits from this portion of the study were from greenhouse-grown plants of the Stoneville 213 cv., except Figure 1. Moore, Stuart, and Mahan pecans from the Texas A&M University farm were used. Okra fruits were from field-grown, United States Department of Agriculture Plant Introduction No. 65.

Fruits of the ages given in the figures were surface-sterilized with commercial grade sodium hypochloride (diluted 1:4) and placed upright in Petri dishes containing a 2% glucose solution. Groups of 10 to 15 fruits were treated with CO_2 in 54.5-liter Plexiglas chambers. Dishes of calcium carbonate were placed in the chambers to remove excess humidity. CO_2 was applied at 0 (control treatment) and 13%. The experiments were conducted in a growth room with a 15-hr photoperiod and a constant temperature of 27 C. Dehiscence was recorded and each chamber was aired and refumigated daily until the completion of dehiscence.

CO_2-ethylene competition was tested by applying both CO_2 and ethylene alone and in various combinations to field-grown, Tamcot SP37 cotton fruits as detailed in Figure 1. All other aspects of the experiment were identical with those described in the preceding paragraph.

Reduced Pressure. The effect of reduced pressure on dehiscence of detached cotton, pecan, and okra fruits was observed. Handling, numbers, and ages of fruits were identical with those described for CO_2 experiments. Fruits in the treatment were held at 200 mm Hg pressure under 12-liter bell jars. In this system the bell jars were placed between the vacuum source and a Matheson No. 49 vacuum regulator which maintained the desired pressure by allowing air to bleed into the system. In order to insure that accumulation of ethylene or depletion of available oxygen was not a factor in the experiments, air flow was maintained through both the vacuum and atmospheric pressure (control) systems. The flow of air passing over the fruits was regulated by a needle valve placed between the vacuum source and the bell jars; air flow was measured by a flow meter placed between the needle valve and the bell jars. The air flow in control chambers in most experiments was near 500 ml/min, providing air exchange each 12 min. In all experiments the chambers were opened daily and the number of fruit showing any opening of the separation zone due to slight pressure was recorded as dehisced.

The effect of reduced pressure (200 mm Hg) on dehiscence of attached cotton fruits was studied by enclosing intact plants in 24-liter chambers made of two 12-liter bell jars placed end to end. Except for chamber size, this system was identical with the one used for detached fruits. Control plants were enclosed in chambers at atmospheric pressure. Two treatment and two control chambers, with one plant per chamber, were used. An observed air flow of about 1000 ml/min was maintained through all chambers providing air exchange in 24 min or less.

[1] This research was supported in part by Cotton Incorporated and National Science Foundation Grant GB-5640. A contribution of the Texas Agricultural Experiment Station.

[2] Present address: Texas A&M University Research and Extension Center at Overton, Drawer E, Overton, Tex. 75684.

Table I. *Dehiscence of Detached Fruits Treated with Air or Air Containing 13% CO_2*

Weeks after anthesis (cotton and okra) and weeks before dehiscence (pecan) indicate the relative stage of development when the fruits were detached.

Time to Dehiscence	Cotton Weeks after Anthesis						Okra Weeks after Anthesis				Pecan Weeks before Dehiscence					
	1 to 2		3 to 4		5 to 6		1		3 to 4		Moore 2 to 3		Mahan 1 to 2		Stuart 1 to 2	
	Air	CO_2[1]	Air	CO_2	Air	CO_2	Air	CO_2	Air	CO_2	Air	CO_2	Air	CO_2	Air	CO_2
days	%															
2	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	6
4	0	0	0	0	50	0	40	0	0	0	0	0	13	25	25	25
6	0	0	0	0	100	0	100	0	0	0	33	0	56	44	25	25
8	0	0	0	0		0		0	0	0	87	20	100	63	38	50
10	10	0	0	0		30[2]		0	0	0	100	73		69	75	75
12	20	0	0	0		100		20	0	0		100		100	94	88
14	20	0	0	0				100	0	0					100	94
16	40	0	10	0					30	0						100
18	50	20	30	0					40	0						
20	50	20	30	0					80	0						
22	50	20	90	0					100	10						
24	70	20	100	0						70						
26	90	20		0						100						
28	100	20[2]		0[2]												

[1] Air containing 13% CO_2.

[2] Removed from 13% CO_2 and placed in 10 μl/l of ethylene on the date indicated.

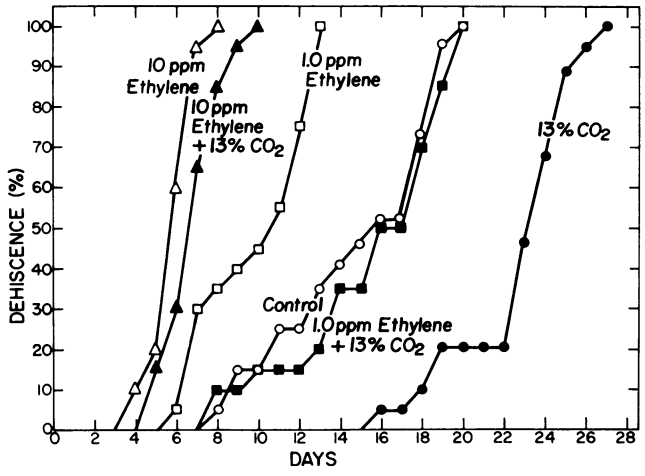

FIG. 1. Competitive effects of CO_2 and ethylene on dehiscence of detached cotton fruits. Field-grown Tamcot SP37 fruits were collected 3 to 5 weeks after bloom and were treated in the dark in groups of 20 fruits each, with the indicated levels of CO_2, ethylene, or both.

The role of oxygen depletion in the effect of reduced pressure on dehiscence was determined. Groups of fruits were placed under 200 mm pressure, atmospheric pressure (control), or 200 mm pressure with a flow of 80% O_2. O_2 content was calculated to be approximately equal in an atmosphere of 80% O_2 at 200 mm pressure and in room air at atmospheric pressure.

RESULTS

Treatment with CO_2. CO_2 delayed dehiscence of all ages of detached cotton, pecan, and okra fruits (Table I). Dehiscence of cotton fruits was so effectively delayed that some of them eventually deteriorated without dehiscing. After 28 days, undehisced cotton fruits from 1 to 2 and 3 to 4 weeks after anthesis were removed from CO_2, and half of them were fumigated with 10 μl/l of ethylene while the other half were left untreated. The younger fruits dehisced 3 to 5 days after being placed in the ethylene atmosphere, while most of the fruits left untreated deteriorated without dehiscing (data not shown). Dehiscence of fruits in the CO_2-treated 3- to 4-week-old group was unaffected by 10 μl/l of ethylene, but the 5- to 6-week-old cotton fruits treated with CO_2, removed from CO_2 after only 10 days, and placed in 10 μl/l of ethylene, completely dehisced after 1 to 2 days. Light was not necessary for the CO_2 delay of dehiscence; the response to CO_2 was demonstrated with detached bolls in the dark. Control greenhouse-grown Stoneville 213 fruits, 5 to 6 weeks after anthesis, completely dehisced in 12 days in the dark, but CO_2-treated bolls reached only 70% dehiscence in 21 days (data similar to those in Fig. 1).

Dehiscence of both 1- and 3- to 4-week-old okra fruits was readily delayed by CO_2 (Table I). Younger okra fruits (detached 1 week after anthesis) dehisced much sooner than fruits detached 3 to 4 weeks after anthesis, a situation already noted with fumigation of okra fruits with ethylene (9).

CO_2 also effectively delayed dehiscence of detached pecan fruits (Table I). Dehiscence of Moore and Mahan pecans was delayed for 2 to 3 days and 1 to 3 days, respectively, by CO_2. Dehiscence of Stuart pecans was not appreciably delayed by CO_2; however, CO_2 damaged Stuart fruits (the outer fruit walls blackened) which probably prevented a delay in dehiscence by CO_2.

Competition between CO_2 and ethylene in the mediation of dehiscence did occur (Fig. 1). Detached cotton fruits treated with 13% CO_2 plus 1 μl of ethylene per liter of air dehisced 5 to 7 days later than fruits treated with 1 μl/l of ethylene alone. CO_2 had much less effect in the presence of 10 μl/l of ethylene. The combination of 13% CO_2 and 1 μl/l of ethylene resulted in a competitive balance since these fruits dehisced at a rate almost identical with the controls. Fruits treated with 13% CO_2 alone dehisced 6 to 8 days after both the control and fruits treated with 13% CO_2 plus 1 μl/l of ethylene.

Table II. *Effect of Atmospheric or Reduced Pressure on the Dehiscence of Detached Fruits*

Weeks after anthesis (cotton and okra) and weeks before dehiscence (pecan) indicate the relative stage of development when the fruits were detached.

Time to Dehiscence	Cotton Weeks after Anthesis						Okra Weeks after Anthesis				Pecan Weeks before Dehiscence			
	1 to 2		3 to 4		5 to 6		1		3 to 4		Moore 1 to 2		Stuart 1 to 2	
	C[1]	V[2]	C	V	C	V	C	V	C	V	C	V	C	V
days	%													
2	0	0	0	0	0	0	0	0	0	0	0	0	19	12
4	0	0	0	0	20	0	70	20	0	0	0	0	25	12
6	0	0	0	0	100	0	100	80	0	0	13	0	44	12
8	0	0	0	0		30		100	0	0	27	0	50	19
10	10	0	0	0		100			0	0	87	13	75	31
12	10	0	0	0					0	0	100	53	100	69
14	10	0	0	0					60	0		100		81
16	30	0	10	0					100	0				94
18	80	0	50	0						20				100
20	80	0	70	0						80				
22	90	0	100	0						100				
24	100	10		50										
26		10		70										
28		20[3]		100										

[1] Atmospheric pressure.

[2] Reduced pressure (200 mm Hg).

[3] Removed from reduced pressure and placed in atmospheric pressure with or without 10 μl/l of ethylene.

Reduced Pressure. Reduced pressure of 200 mm Hg effectively delayed dehiscence of detached fruits of cotton, okra, and pecan in a manner similar to the response to CO_2. Dehiscence of 1- to 2-, 3- to 4-, and 5- to 6-week-old cotton fruits was delayed considerably at 200 mm pressure (Table II). The two younger ages of cotton fruits dehisced more slowly than the older fruits. The 1- to 2-week-old control fruits were restored to atmospheric pressure after 28 days; and half the fruits fumigated with 10 μl/l of ethylene dehisced after 3 to 4 days while the other half (untreated) eventually deteriorated without dehiscing (data not shown). Reduced pressure delayed dehiscence of young okra fruits 1 to 2 days, and 3- to 4-week-old okra fruits approximately 5 days (Table II). Reduced pressure also effectively delayed dehiscence of pecan fruits (Table II).

Tests with intact cotton plants with attached maturing fruits revealed that reduced pressure delayed dehiscence of intact fruits for 3 to 6 days (Fig. 2). Of 18 fruits on the two control plants, dehiscence occurred from 4 to 20 days after the treatment was begun. Plants under reduced pressure had 21 intact fruits and these dehisced from 8 to 25 days after the treatment was started. All of these fruits were from 20 to 36 days after anthesis when the experiment was begun.

The possibility that O_2 depletion, rather than removal of ethylene, caused the delay of dehiscence at 200 mm Hg pressure was investigated. However, cotton fruits that were under 200 mm pressure and 0.21 atm of O_2 (purging gas 80% O_2) dehisced only about 1 day faster than fruits under 200 mm pressure and 0.055 atm of O_2 (purging gas room air). Fruits in the control dehisced much sooner than either of the treatments under reduced pressure. Thus, O_2 depletion had little effect on dehiscence of fruits at 200 mm pressure.

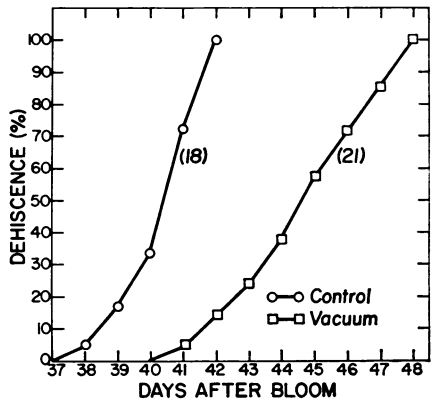

FIG. 2. Effect of reduced pressure on dehiscence of intact fruits of cotton. Two greenhouse-grown Stoneville 213 plants with maturing fruits were held at 200 mm Hg. Control plants were under atmospheric pressure. The total number of fruits involved in each treatment is in parentheses. Data are plotted as a cumulative percentage of fruit dehiscence relative to the number of days from anthesis (bloom) to dehiscence of each fruit. Bloom occurred over a 16-day period; thus, the data were adjusted to the relative age from anthesis to dehiscence of each fruit.

DISCUSSION

CO_2 has been shown to be a competitive inhibitor of ethylene action (6). Thus, if ethylene produced by the fruit causes dehiscence, then fumigation with CO_2 should delay dehiscence. This was, in fact, the result of exposure of cotton, okra, and pecan fruit to 13% CO_2 (Table I). The only exception was the failure of CO_2 to delay dehiscence of Stuart pecan fruits. This failure was attributed to a visible toxicity, blackened fruit walls, produced by the CO_2.

Further evidence that CO_2 and ethylene do compete in their effects on fruit dehiscence was provided by exposure of cotton fruits to mixtures of the gases (Fig. 1). Fruits fumigated with 10.0 and 1.0 μl/l of ethylene dehisced sooner than fruits that were fumigated with 13% CO_2 mixed with 10.0 and 1.0 μl/l of ethylene. CO_2 more effectively delayed dehiscence of fruits

treated with 1 μl/l of ethylene than 10 μl/l of ethylene, an observation consistent with the kinetics of CO_2-ethylene competition (4). The combination of 13% CO_2 and 1.0 μl/l of ethylene resulted in a competitive balance in which fruits dehisced at the same rate as fruits in the control. Cotton fruits, after long exposure to CO_2, were readily stimulated to dehisce by ethylene (data not shown). This result indicates that the effects of CO_2 were reversible and that, in general, detached fruits did not rapidly lose their ability to respond to ethylene with time after they were detached. However, some fruits eventually reached a state of deterioration in which they would not respond to ethylene. These observations also support the concept that ethylene must exceed a certain threshold level before it can initiate dehiscence.

Reduced atmospheric pressure (200 mm Hg), employed to accelerate escape of ethylene from detached fruits of cotton, pecan, and okra, delayed dehiscence of all fruits (Table II, Fig. 2). As a rule, the response of fruits to reduced pressure was much the same as the response to CO_2, with the possible exception that dehiscence of pecans was delayed longer by reduced pressure (Table II) than by CO_2 (Table I). This result agrees with the very high ethylene production rate and internal concentrations for pecan fruits (9). Possibly a level of ethylene would soon be reached that would overbalance the effect of CO_2, while reduced pressure could keep the internal level of ethylene below the threshold level for a longer time.

Ethylene removal was the major reason for the delay in dehiscence of fruits under reduced pressure. Since low O_2 prevents ethylene from acting on tissues (7), the slightly increased rate of dehiscence by fruits that were under 200 mm pressure with added O_2 (partial pressure of 0.021 atm) was possibly a result of increased action of the ethylene present in the fruits.

Reduced pressure did not appear to have a detrimental effect on the ability of fruits to respond to ethylene. Young cotton fruits that had been under 200 mm pressure for 28 days were restored to atmospheric pressure and were stimulated, by 10 μl/l of ethylene, to dehisce in 5 days (data not shown). This was approximately the same rate of dehiscence observed with fruits placed in 10 μl/l of ethylene soon after they were detached.

It might be asked why fruits treated with CO_2 or pressure reduction eventually dehisce anyway. In fact, some never do, since after several weeks they eventually decay, but, up to that time, they can respond to ethylene. These circumstances simply re-emphazie the juvenile-senescene hormone balance concept of fruit dehiscence regulation. As the fruits age they produce more and more ethylene until eventually the minimum concentration for initiation of dehiscence is exceeded (9). Thus, at this time, production of ethylene is occurring at a rate greater than can be compensated for by CO_2 or vacuum. The data from both detached and intact fruit (Table II, Fig. 2) indicate that ethylene production eventually exceeds the threshold level for induction of dehiscence and reduction of natural juvenile factors may play a role in the over-all process.

Quantitative relationships can be established for mature fruit by using production rate data, the ratio of internal ethylene to the rate of production, the dehiscence dose-response relationship (9) and the K_I for ethylene-CO_2 competition (6) or the percentage removal of ethylene by 200 mm Hg pressure (4). Such calculations are complicated because the data are from both attached and detached fruits from several populations, and one must assume that the CO_2 and reduced pressure treatments do not substantially alter ethylene production rates. However, such calculations indicate that both treatments would delay the time required for ethylene levels to exceed threshold levels and that natural production would eventually approach or exceed saturation levels, thus completely escaping the dehiscence-blocking action of the treatments. The results agree with these projections. No calculations were made for the young fruit because data were not available on their ethylene production rates after lengthy periods of detachment or on their production rate to internal level relationships. However, young expanding cotton fruit produce relatively low levels of ethylene (9, 10) and most of these fruits, predictably, were prevented from dehiscing for the duration of their storage life by both CO_2 (1- to 2- and 3- to 4-week-old fruits, Table I) and reduced pressure (1- to 2-week-old fruits, Table II).

LITERATURE CITED

1. Abeles, F. B. and H. E. Gahagan. 1968. Abscission: The role of ethylene, ethylene analogues, carbon dioxide and oxygen. Plant Physiol. 43: 1255-1258.
2. Burg, S. P. and K. V. Thimann. 1959. The physiology of ethylene formation in apples. Proc. Nat. Acad. Sci. U. S. A. 45: 335-344.
3. Burg, S. P. and E. A. Burg. 1965. Ethylene action and the ripening of fruits. Science 148: 1190-1196.
4. Burg, S. P. and E. A. Burg. 1965. Gas exchange in fruits. Physiol. Plant. 8: 870-884.
5. Burg, S. P. and E. A. Burg. 1966. Fruit storage at sub-atmospheric pressures. Science 153: 314-315.
6. Burg, S. P. and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42: 144-152.
7. Hansen, E. 1942. Quantitative study of ethylene production in relation to respiration of pears. Bot. Gaz. 103: 543-558.
8. Kidd, F. and C. West. 1945. Respiratory activity and duration of life of apples gathered at different stages of development and subsequently maintained at constant temperature. Plant Physiol. 20: 467-504.
9. Lipe, J. A. and P. W. Morgan. 1972. Ethylene: Role in fruit abscission and dehiscence processes. Plant Physiol. 50: 759-764.
10. Morgan, P. W., D. L. Ketring, E. M. Beyer, Jr., and J. A. Lipe. 1972. Functions of naturally produced ethylene in abscission, dehiscence and seed germination. *In:* D. J. Carr, ed., Plant Growth Substances 1970, Springer-Verlag, New York. pp. 502-509.