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Autoinhibition of Ethylene Formation in Nonripening Stages of the Fruit of Sycomore Fig (*Ficus sycomorus* L.)

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M. ZERONI AND J. GALIL
Department of Botany, Tel Aviv University, The Dr. George S. Wise Center for Life Sciences, Tel Aviv, Israel
S. BEN-YEHOSHUA
The Volcani Institute for Agricultural Research, Bet-Dagan, Israel

ABSTRACT

Differences in the mechanism of ethylene emanation of *Ficus sycomorus* L. during various stages of the fruit development were investigated by enclosing the figs in jars. Two distinct patterns of ethylene emanation were found. Pattern a. in stages not capable of ripening, neither spontaneously nor as a result of physiological treatment (nonripening stages A and C), ethylene concentration in the jar increased linearly for a short time and then remained constant. Pattern b. in stages capable of ripening (ripening stages B, D, and E), the linear increase in ethylene concentration continued for the entire period of measurement. In nonripening stages, ethylene emanation stopped when ethylene concentration in the jar reached a constant value (0.6 μl/l at stage C). Aeration of the figs and the jar renewed ethylene emanation. CO_2 concentration in the jar never exceeded 0.5%. Treatment of stage C figs with 0.6 to 10 μl/l exogenous ethylene caused immediate and complete cessation of ethylene emanation whereas the same treatment did not cause any change in rate of ethylene emanation from figs at the ripening stages B and D. Gashing (wounding) of stage C figs temporarily changed the pattern of ethylene emanation from pattern *a* to pattern *b*.

We concluded that in the nonripening stages ethylene acts as an autoinhibitor of its own production but this does not occur in the ripening stages.

Galil and Eisikowitch (4) divided the developmental course of the sycomore fig into five stages: stage A (prefemale): young fig prior to opening of the ostiole, stage of cell division, quick growth, and differentiation of the female organs; stage B (female): ostiolar scales loosen, female flowers mature, the impregnated females of the wasp *Sycophaga sycomori* penetrate into the syconia and oviposit in the ovaries of female flowers, growth rate slows down, wounding (gashing) of the fig at this stage causes ripening within 3 days; stage C (interfloral): ostiolar scales retighten, fruit develops, wasp larvae develop within the ovaries, thus transforming them into galls, stage of slow growth, all fruit cavities full with latex; stage D (male): male flowers mature, wasps reach the imago stage, fertilized females emerge from their galls and enter the syconial cavity, later they leave the fig via tunnels bored by the males, rapid growth and beginning of ripening; stage E (postfloral): continuation of ripening and rapid growth.

Ripening stages are defined as developmental stages during which the fruits are capable of ripening either spontaneously or as a result of physiological treatment (stages B, D, and E). Nonripening stages are stages which are not capable of ripening under the above mentioned conditions, but occasionally ripening may be enforced by harsh treatment (stages A and C).

The process whereby a fruit at a nonripening stage of development becomes ready to ripen is not well understood. It is known that as the fruit matures, ethylene production is accelerated suddenly, and brings about other events such as a climacteric rise of respiration, a softening of the fruit and a change in its color and taste. Pratt and Goeschl (9) divided the climacteric fruit into two main types: *(a)* fruits which contain during their nonripening stages enough ethylene to trigger the ripening process although this does not happen until a certain stage of maturity has been attained, and *(b)* fruits which do not contain enough ethylene to begin ripening so that an increase in ethylene formation must precede ripening. Burg and Burg (3) showed that the sensitivity of fruit to ethylene changes with the age of the fruit. It increases towards the ripening stages so that a concentration of ethylene ineffective in nonripening stages becomes effective in the ripening ones.

Sycomore fig (*Ficus sycomorus* L.) does not seem to contain enough ethylene during its nonripening stages for commencement of ripening, and ethylene emanation rises before ripening takes place; the sensitivity of the fruit to ethylene is higher in the ripening stages than in the nonripening ones (15).

The aim of this work was to study the differences in the mechanism of ethylene emanation during different stages of the fruit development in sycomore fig and to learn what causes the rise in ethylene production towards ripening and what makes it so abrupt.

MATERIALS AND METHODS

The experiments were carried out on figs (syconia) of *Ficus sycomorus* L. var. Balami growing on the campus of Tel Aviv University. Syconia were labeled when the fig emerged from the bud scales. Subsequently, a sample of figs of known age and developmental stage was harvested when desired. The figs were fully inhabited by larvae of the wasp *Sycophaga sycomori* L. (Chalcidoidea, Torymidae), thus more than 95% of the female flowers were transformed into galls during phase c.

Ethylene emanation was determined according to Burg and Burg (2) by enclosing fruit of known age and developmental stage in jars for 1 to 2 hr.

In most experiments, jars of a standard volume of 45 ml were used. In some, however, the figs were enclosed in larger jars of 150 ml and 270 ml. Gas samples of 1 ml were withdrawn from the jars every 15 min (or every 5 min for phase c). The samples were injected into a Packard gas chromatograph equipped with a column (183 x 164 cm) packed with 80 to 100 mesh alumina and connected to a flame ionization detector. The instrument could detect 10 nl/l ethylene in a 1-ml sample.

The amount of ethylene production attributable to wounding during harvest was estimated by comparing ethylene emanation from untreated excised fruit to that of fruit in which silicone grease (SISS, France) was applied to the cut surfaces of the

petioles. The difference was found to be less than 0.5% for all stages and therefore this kind of treatment was discarded.

CO_2 emanation was determined according to Tadmor *et al.* by injecting the sample into a column of 80 to 100 mesh Porapak Q (Waters Manufacturing Inc., Wayland, Mass.) connected to a thermal conductivity detector. The sample was then passed into the alumina column for determination of ethylene (5).

Ethylene concentration in the jars was standardized in two steps. At first a concentrated ethylene atmosphere was prepared by injecting a known amount of pure ethylene (Gordon Inc., Tel Aviv), with a gas-tight syringe, into vials of known volume sealed by self-adhesive rubber. The gas within each vial was mixed with the aid of a Teflon-coated magnet. Ultimately a desired volume of this gas was injected into the fruit containing jars. Aeration of the jars and the figs was effected by taking the fruits out of the jar and blowing an air stream of 500 ml/min over the fruits and into the jar for 5 min.

Gashing (wounding) was performed on attached figs by plunging a scalpel downwards into the fig cavity to produce 11 mm long cuts on the surface. After gashing, the figs were harvested at 1-hr intervals for 27 hr and immediately enclosed in jars for ethylene emanation tests. Experiments were repeated three to four times, with three to ten jars per test and two to five figs in each jar.

RESULTS

Two distinct patterns of ethylene emanation were found when figs at different developmental stages were enclosed in 45 ml jars. *(a)* Ethylene concentration increased linearly for a short time and then remained constant. This pattern was found in figs at stages A and C (Figs. 1 and 2). *(b)* Ethylene concentration increased linearly throughout the measurement (2 hr). This pattern was found in figs at stages B, D, and E (Fig. 3).

Between the 2nd and the 7th day of development of the figs at stage A, ethylene emanation ceased 30 min after the figs were enclosed in the jar. Between the 8th and the 15th day—the last days of stage A—ethylene concentration in the jar continued to increase for a short time after the initial 30 min of enclosure, but at a slower rate (Fig. 1). Figs at stage C stopped emanating ethylene after 15 min and the final concentration was relatively low (0.6 μl/l) (Fig. 2). Here, too, on the last day of stage C (the 38th day of development), ethylene concentration in the jar increased at a slower rate after the first 15 min of enclosure and then ceased.

CO_2 accumulated steadily and linearly throughout the measurement for all the stages tested (Table I). The concentration of CO_2 in the jars when ethylene emanation stopped was less than 1% for stage A and less than 0.5% for stage C. No significant differences have been found between the inhabited figs of stage C and the uninhabited ones of stages A, B, and B gashed throughout the measurement. A steep rise in CO_2 concentration in the jars was found only in the climacteric stage D.

When stage C figs were enclosed in larger test jars (150 ml and 270 ml rather than 45-ml jars), ethylene emanation continued until the same final concentration was reached (0.600 ± 0.054 μl/l) as in the first experiments, and then stopped. The duration of the ethylene concentration rise increased, depending upon the volume of the jar (Table II).

The pattern of ethylene emanation of gashed B figs was the same as that of intact ones. Rates of emanation remained constant throughout the test period of 120 min (Fig. 3). Figs harvested 1 hr after gashing emanated 50-fold more ethylene than ungashed figs and ripened within 3 days.

Gashing of C stage figs temporarily changed their pattern of

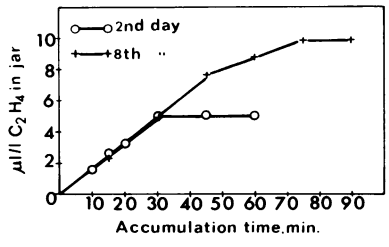

FIG. 1. Accumulation of ethylene in a 45-ml jar containing stage A figs.

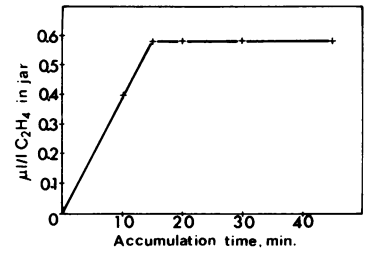

FIG. 2. Accumulation of ethylene in a 45-ml jar containing stage C figs.

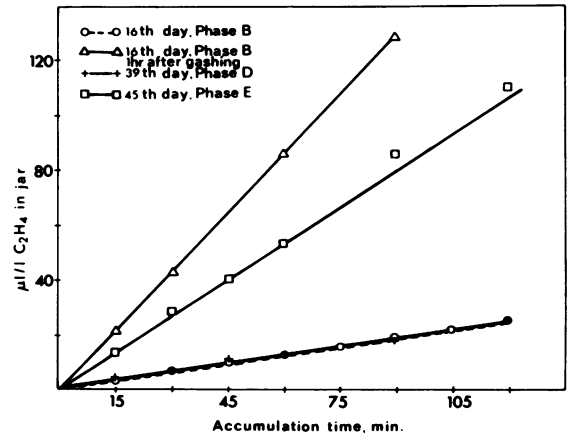

FIG. 3. Accumulation of ethylene in a 45-ml jar containing figs at stages B, D, E, and gashed B.

Table I. *CO_2 Accumulation during Enclosure of Figs of Various Ages and Stages of Development in 45-ml Test Jars*

Stage	Day of Development	CO_2 Accumulation in Jar (min after enclosure)					
		5	10	15	20	30	60
				%			
A	3			0.53		0.92	2.36
A	8			0.51		0.82	1.97
B[1]	16			0.36		0.81	1.47
B gashed	17			0.46		0.73	1.93
C	22	0.16	0.29	0.47	0.61	0.91	2.01
C	30	0.13	0.32	0.40	0.55	0.95	1.67
D	39			2.57		4.95	8.18

[1] Uninhabited figs.

Table II. *Effect of Jar Volume on Final Ethylene Concentration and Duration of Its Linear Emanation by Stage C Figs*

Volume of Jar	Final Ethylene Concn	Time of linear Rise of Conc
ml	*µl/l*	*min*
45	0.600 ± 0.054	15
150	0.598 ± 0.052	35
270	0.602 ± 0.055	90

ethylene emanation. Figs harvested within the first 6 hr after gashing, produced linear rise of the ethylene concentration in the jar throughout the 120 min of the test. In figs harvested 12 hr after gashing, ethylene emanation slowed down 30 min after their enclosure in jars. Starting from the 24th hr after gashing the ethylene emanation ceased 15 min following enclosure of the figs (Fig. 4), as in ungashed figs. Gashing did not cause ripening of stage C figs.

A 5-min aeration of the jars after the enclosed C stage figs ceased emanating ethylene, renewed the ethylene production. Rate of emanation by the aerated figs equalled that by initially enclosed figs. The time at which ethylene production in the closed jar ceased and the final concentration attained were also equal for figs enclosed for the first time and for ones re-enclosed after aeration. A second and third aeration gave the same results, but following a fourth aeration (*i.e.* after a fifth enclosure of the figs and more than 100 min following their harvesting) the rate of emanation became erratic (Table III).

Figs at stages B, gashed B, and D showed no change in the pattern or rate of ethylene emanation when enclosed in an atmosphere containing 1, 2, 5 or, 10 μl/l ethylene compared to controls. A or C stage figs that were enclosed in an atmosphere containing 0.6 (for stage C), 1, or 2 μl/l ethylene did not emanate any ethylene, the concentration of ethylene within the jar remaining constant. Stage A and C figs enclosed in an atmosphere containing 5 or 10 μl/l ethylene, at first absorbed a part of the ethylene from the surroundings so that the ethylene concentration in the jar decreased somewhat, but no further change occurred subsequently and the mean concentration remained constant (Fig. 5). Fluctuations in ethylene concentrations were high and reached 20% (SE ± 0.12) after application of 5 and 10 μl/l ethylene. CO_2 concentrations did not change comparing to control and equalled that staged in Table I.

DISCUSSION

No evidence was found for the occurrence of autocatalysis of ethylene in the ripening stages of the fruit of *Ficus sycomorus*. There was no exponential rise in the rate of ethylene emanation in the test jar, such as was found for apple fruit (10). Ethylene application to nonripening fruit did not cause ripening except in an extremely high concentration.

The present study points to a qualitative difference in the type of ethylene emanation between fruits at ripening and nonripening stages of development. In the nonripening stages (A and C), ethylene emanation ceased after a brief period of time. That ethylene itself was responsible for inhibiting the ethylene emanation from figs in nonripening stages is clearly indicated from the following findings. *(a)* Aeration of nonripening syconia restored the ethylene production at the regular rate, suggesting that the inhibition is not related to senescence of the fruit tissues or to the wound caused by harvest, but rather to a volatile substance accumulated in the jar. *(b)* Increasing the volume of the jar merely prolonged the time required for achieving equilibrium but did not affect the ethylene emanation proper nor the concentration at which it stopped. This suggests that the concentration of the gases within the jar was related to the cessation of ethylene production. *(c)* Gashing (wounding) of the fruit tissue in the nonripening stage C temporarily changed the pattern of ethylene emanation. *(d)* CO_2 concentration in the jar never exceeded 0.5% at stage C and 1% at stage A when ethylene emanation stopped. Moreover, cessation of ethylene emanation occurred only in preclimacteric stages, whereas in climacteric stages, especially at stage D, in which respiration is much faster, ethylene emanation continued linearly for a long period of time. This shows that neither CO_2 accumulation (1, 7, 8) nor O_2 depletion (3) are responsible for the phenomenon. *(e)* Treatment with 0.6 to 10 μl/l exogenous ethylene caused an immediate and complete cessation of ethylene emanation by the nonripening stages of fruit tested, whereas the ripening stages continued emanating ethylene linearly after the same treatment.

From all the above, we concluded that ethylene acts as an inhibitor of its own production in nonripening stages of the sycomore fruit while no autoinhibition of ethylene is found in the ripening stages. The fact that exogenous ethylene at the proper concentration immediately stopped ethylene emanation in nonripening stages tends to exclude the possibility that another gas that may have emanated together with ethylene inhibited the ethylene production.

Preliminary tests conducted by the present authors have

Table III. *Influence of 5-min Aeration and Repeated Enclosures on Ethylene Production by Stage C Figs*

Time after harvest	No. of Enclosure	Time after Enclosure	C_2H_4 Concn in Jar
min		*min*	*μl/l*
5	1	5	0.21 ± 0.015
10		10	0.38 ± 0.028
15		15	0.59 ± 0.056
20		20	0.60 ± 0.053
30	2	5	0.20 ± 0.014
35		10	0.41 ± 0.030
40		15	0.59 ± 0.058
45		20	0.58 ± 0.057
55	3	5	0.19 ± 0.015
60		10	0.40 ± 0.028
65		15	0.61 ± 0.052
70		20	0.60 ± 0.050
80	4	5	0.20 ± 0.012
85		10	0.41 ± 0.029
90		15	0.61 ± 0.050
95		20	0.60 ± 0.051
105	5	5	0.18 ± 0.074
110		10	0.48 ± 0.21
115		15	0.74 ± 0.53
120		20	0.56 ± 0.54

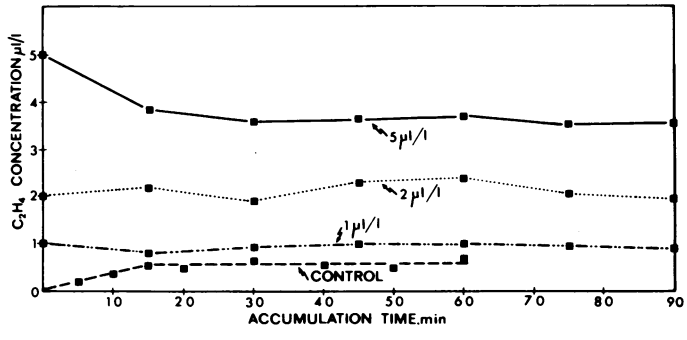

FIG. 5. Effect of ethylene-containing atmosphere on ethylene accumulation in jars with stage C figs.

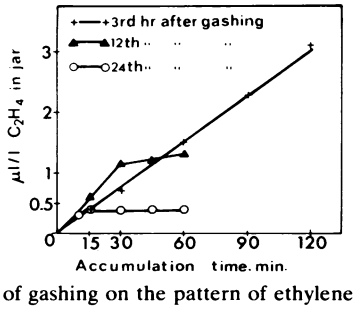

FIG. 4. Effect of gashing on the pattern of ethylene emanation from stage C figs.

shown similar differences in the response to ethylene of developing tomato fruit: ethylene emanation by the green fruit was autoinhibited while that by ripening fruit was not.

Vendrell and McGlasson (13) found that a temporary treatment of preclimacteric banana fruit slices with 5 to 10 μl/l exogenous ethylene partly suppressed the subsequent endogenous ethylene production of these tissues, after the exogenous ethylene had been removed and once the ripening process was induced; they called this phenomenon autoinhibition of ethylene. A similar finding was reported by Zauberman and Fuchs (14) in chilled avocado fruit. This phenomenon fails to explain the differences observed by us between the ripening and the nonripening stages of the fruit, since the exogenous ethylene induced ripening of the banana slices despite the decrease in endogenous ethylene production and because high ethylene concentration does not exist in nonripening stages of the fruit.

The present study shows that the autoinhibition occurs immediately upon the application of exogenous ethylene and continues for as long as exogenous ethylene is available. The interesting point is that the autoinhibition exists only in the nonripening stages of the fruit and not in the ripening stages. This finding allows us to conjecture regarding the transformation of sycomore fruit from nonripening to ripening stages. It is possible that in nonripening stages of this fruit, the autoinhibitory phenomenon prevents the fruit from accumulating a sufficient amount of ethylene to trigger the ripening process. Treatment with exogenous ethylene at a concentration insufficient to cause ripening, stops ethylene production by the figs and thereby prevents the onset of ripening. At a certain stage this autoinhibitory phenomenon gradually weakens and finally disappears, and consequently the ethylene concentration in the tissue rises to a level sufficient to induce ripening.

Admittedly, the enclosure of figs in jars is an artificial manipulation, whereas the fruit in nature are ordinarily exposed to the air and the ethylene emanated by them is freely dispersed. However, every ethylene-producing cell is surrounded by intercellular spaces from which ethylene emerges by diffusion only. Insofar as the diffusion is a slow process and its rate depends on ethylene concentration inside the fruit, ethylene may indeed accumulate around the cells. In nonripening stages, a rise in the ethylene concentration in the intercellular spaces is likely to cause the cessation of ethylene production and consequently a low mean emanation rate. In ripening stages on the other hand, where autoinhibition of ethylene does not occur, ethylene production does not cease and its mean emanation rate is high.

There may indeed be different biochemical pathways of ethylene production during different stages of fruit development, as suggested by Burg and Burg (3). Spencer (11) similarly maintains that in several organisms ethylene formation may occur via different pathways during different stages of their development.

Hall *et al.* (6) reported that Ethephon application affected grapes differently at various stages of their development. During the second half of the first rapid growth phase or at the start of the slow growth phase of berry development, Ethephon delayed ripening, whereas at more advanced phases of development of the same fruit, it hastened the onset of ripening.

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