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# Ethylene Evolution following Treatment with 1-Aminocyclopropane-1-carboxylic Acid and Ethephon in an *in Vitro* Olive Shoot System in Relation to Leaf Abscission

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## ABSTRACT

**1-Aminocyclopropane-1-carboxylic acid (ACC) supplied via the cut base of detached olive shoots caused a burst of ethylene from leaves, but other cyclopropanes tested did not exhibit this effect. Ethephon (ET) and another ethylene-releasing compound caused a prolonged increase in ethylene evolution. ACC had only a very limited effect on leaf abscission regardless of concentration, whereas shoots placed with cut bases in ET for 60 to 80 minutes exhibited 100% leaf abscission within 90 hours. Shoots with inflorescences treated with ET just prior to anthesis began to wilt *in vitro* within 20 to 30 hours and failed to exhibit leaf abscission. At earlier stages of development, ET induced more leaf abscission on reproductive shoots than on vegetative shoots. It is suggested that the duration of ethylene evolution from the leaves governs their potential for abscission and that bursts of ethylene evolution even though large in amount may not induce abscission.**

Ethylene had been shown to be an important senescence (1) and abscission (1, 11, 14) promoting agent. Both ethylene gas (5, 12) and ethylene-releasing compounds (13, 16, 21) have been used in abscission studies. As these compounds have been generally applied exogenously, the metabolic response depended on penetration and, with the ethylene-releasing compounds, on a non-metabolic degradation (6, 15). Until recently, known metabolic precursors of ethylene did not significantly increase ethylene evolution. The recent work of Adams and Yang (3) described the role of ACC[2] in the pathway of ethylene biosynthesis. This last step precursor of ethylene, unaffected by AVG, is readily metabolized (9, 23) to ethylene and application of ACC to various plant tissues results in a rapid increase in ethylene evolution (10).

Endogenous ethylene evolution, as well as that induced by an ethylene-releasing compound (ET), in relation to abscission of various olive organs, has been established (19). In previous studies (17, 18) we introduced an *in vitro* system in which ET was fed to the shoots via the cut base and moved in the transpiration stream. In these studies we reported abscission induced by ET to be concentration and application time-dependent. Under the conditions reported, leaf abscission was induced, whereas inflorescences or fruits were less affected.

In the present study the potential of olive tissue to metabolize ACC and its relation to abscission was determined. As well, the ability of olive organs to utilize ACC for the induction of abscission was compared to ET, an ethylene-releasing compound.

## MATERIALS AND METHODS

Annual "Manzanillo" olive shoots were collected and the leaves below the first 14 to 16 mature nodes removed. The cut surface was renewed when introducing the shoots into test solutions and the excess length of the defoliated part removed (17). Shoot bases were introduced into 6-ml vials 5-cm high containing 2 ml test solution. The shoots were kept at room temperature and a 14-h light cycle in the laboratory at 7.0 $\mu$E $m^{-2}$ $s^{-1}$ light intensity. The volume of solutions was readjusted with water as required. Only cuttings with mature leaves were taken (19) and in some experiments inflorescences were present. For dark treatments a black cloth tent was employed. If not otherwise cited, the lowest pair of leaves of each shoot were used to determine ethylene evolution. The leaves tested were excised and enclosed, individually, for 2 h in 40-ml vials as described previously for ethylene analysis (17). Abscission was determined following application of a slight downward manual pressure to the leaves or inflorescences. Each treatment was replicated four times using leaves from different shoots. ACC was purchased from Calbiochem. If not cited otherwise, a concentration of $2 \times 10^{-3}$ M was applied. Other cyclopropyls such as CA, CCA, CMK, CMC, and CC were all used at $2 \times 10^{-3}$ M concentration. ET and AL were used at a concentration of $10^{-3}$ M.

## RESULTS

Ethylene evolution from "Manzanillo" leaves and stems of two-leaf cuttings fed with ET and various cyclopropane derivatives was determined. Significant increases in ethylene evolution were found 1 h after ET, ACC, and CMC treatments (Table I). Leaves from ACC-treated cuttings initially evolved much more ethylene than leaves from cuttings treated with ET and CMC. By 3 h, leaves of ET treatments evolved massive quantities of ethylene while significant but comparatively less amounts resulted from ACC, CCA, and CMK treatments (Table I). Stems monitored 3 h after treatment also evolved massive quantities of ethylene from ET with much less from ACC and CMK treatments (Table I).

When 40-cm long shoots were fed for 1 or 2 h, the basal leaves responded to the chemicals similar to the leaves on the two-leaf cuttings, though the level of ethylene evolution was greater than on the two-leaf cuttings (*cf.* Tables I and II). Ethylene evolution induced by ET was still significantly greater than that of control leaves even after 70 h. Leaves on ACC-fed shoots evolved ethylene

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[2] Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; AVG, aminoethoxyvinylglycine; ET, ethephon; CA, cyclopropylamine; CCA, cyclopropanecarboxylic acid; CMK, cyclopropylmethylketone; CMC, cyclopropylmethylcarbinol; CC, cyclopropylcarbinol; AL, 2-chloroethyl-Tris-(2-methoxyethoxy)-silane.

Table I. *Ethylene Evolution from "Manzanillo" Olive Leaves on "Two Leaf" Cuttings Exposed to ET or Various Cyclopropane Compounds*

Mean leaf weight was 270 mg.

| Treatment $2 \times 10^{-3}$ M | Ethylene Evolution | | |
|---|---|---|---|
| | Leaves | | Stem |
| | 1 h | 3 h | 3 h |
| | *nl/10 leaves or stems·h* | | |
| Control—$H_2O$ | 2.5 | 2.7 | 4.9 |
| ET | 10.7 | 74.0 | 177.4 |
| ACC | 36.6 | 11.8 | 24.5 |
| CA | 3.0 | 3.3 | 6.5 |
| CCA | 2.5 | 5.5 | 6.1 |
| CMK | 3.7 | 5.0 | 13.7 |
| CMC | 6.9 | 1.9 | 6.6 |
| CC | 3.5 | 1.44 | 4.5 |
| MSE ± | 1.30 | 1.1 | 0.9 |

Table II. *Effect of Various Cyclopropane Compounds on Ethylene Evolution from Leaves on 40-cm Long Excised "Manzanillo" Olive Shoots*

Mean leaf weight was 235 mg.

| Treatment | Time of application | Ethylene Evolution | | | |
|---|---|---|---|---|---|
| | | Time after transfer to water | | | |
| | | 0 h | 17 h | 41 h | 70 h |
| | *h* | *nl/10 leaves·h*[a] | | | |
| Control—$H_2O$ | 1 | 2.7a | 3.7a | 2.2a | 3.3a |
| ET | 1 | 72.3b[b] | 42.1b | 32.0e | 27.4e |
| ACC | 1 | 61.9b | 3.7a | 3.0a | 1.4a |
| Control—$H_2O$ | 2 | 3.7a | 2.5a | 4.6a | 2.0a |
| ET | 2 | 201.3c[c] | 176.0f | 92.2bd | 27.9e |
| ACC | 2 | 136.8d | 1.9a | 3.3a | 1.3e |

[a] Means followed by the same letter are not significantly different (Duncan's Multiple Range Test, 5%).

[b] 50% leaf abscission after 120 h.

[c] 50% leaf abscission after 80 h.

at levels similar to controls 17 h after feeding. None of the other cyclopropanes tested induced ethylene evolution from leaves above the control level. Leaf abscission occurred only on the ET-fed shoots. In the 1-h treatment, 50% abscission occurred after 120 h, whereas in the 2-h treatment 50% abscission was achieved 40 h earlier. ACC and ET, fed to the shoots for 1 h at various concentrations, induced ethylene evolution from the leaves in proportion to concentration used (Fig. 1). After 1 h, ethylene evolution from leaves on ACC-fed shoots was greater than from leaves on ET-fed shoots. Twenty h after the pulse feeding, however, the elevated ethylene evolution due to ACC diminished at all concentrations fed while leaves on ET-fed shoots continued to evolve large quantities of ethylene (Fig. 1). Feeding $2 \times 10^{-3}$ M ACC for 80 min induced only 21% leaf abscission 120 h after the treatment, whereas a similar treatment with ET induced 100% abscission within 96 h. Only lower leaves abscised on ACC-treated shoots. Higher ACC concentrations did not induce a significantly higher abscission.

Application of $2 \times 10^{-3}$ M ACC or ET for various periods of time resulted in a higher ethylene evolution from leaves on shoots treated with ACC than with ET at the end of each application period (Fig. 2). A high correlation occurred between duration of application and ethylene evolution. The degradation of ACC to ethylene was rapid and complete, whereas that of ET was slower

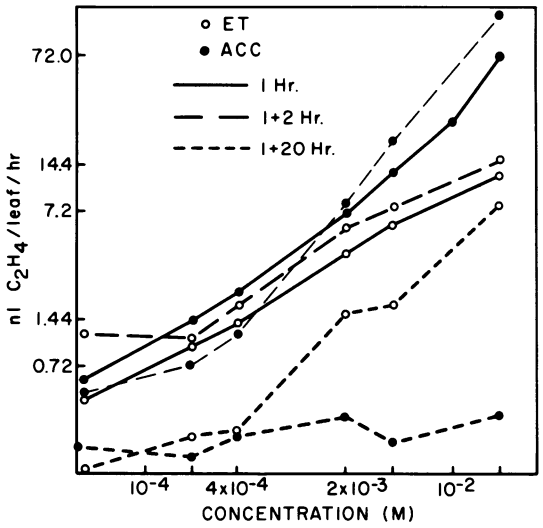


FIG. 1. The effects of ACC and ET concentrations on ethylene evolution from "Manzanillo" olive leaves following pulse feeding through cut bases of excised shoots. (Mean leaf fresh weight 253 mg.)

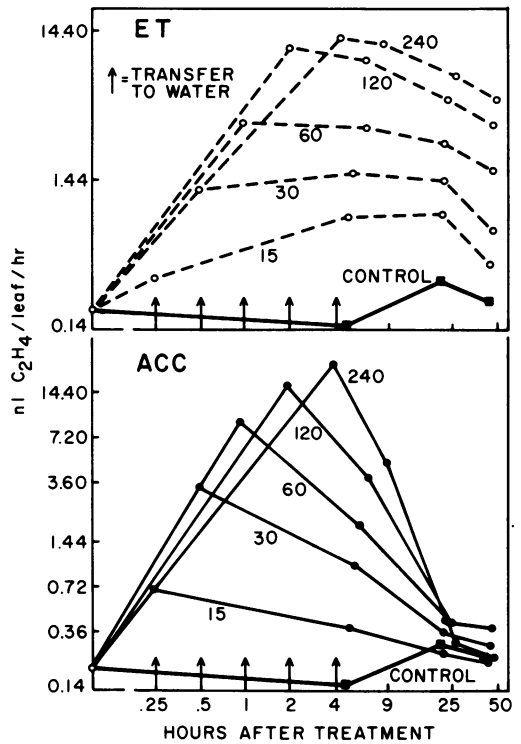


FIG. 2. The effect of application time of ACC and ET on ethylene evolution from "Manzanillo" leaves on excised shoots. Shoots were fed from 15 to 240 min then transferred to water (↑). Results are expressed on a log + log scale. Concentration of chemicals $2 \times 10^{-3}$ M. (Mean leaf fresh weight 271 mg.)

and ethylene evolution remained high even 50 h after the transfer to water. When stem segments from the treated shoots were tested for their ethylene evolution as late as 8 days after the transfer to water a high ethylene evolution from the ET-treated shoot segments was found and could be correlated with the original exposure time (Fig. 3). The stem segments from ACC-treated shoots evolved similar amounts of ethylene to control shoots 8 days after treatment. Leaf abscission due to $2 \times 10^{-3}$ M ACC treatments never exceeded 30%, whereas 100% abscission occurred when ET was applied longer than 60 min.

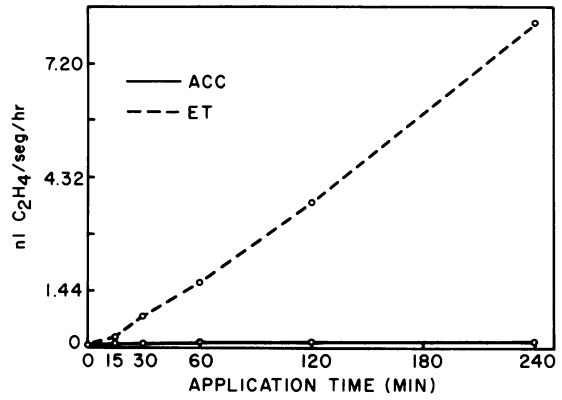


FIG. 3. Ethylene evolution from "Manzanillo" stem segments of shoots 8 days after feeding with ACC and ET for various periods of time. (Concentration of ACC and ET $2 \times 10^{-3}$ M, segments 8-cm long, mean fresh weight 750 mg.)

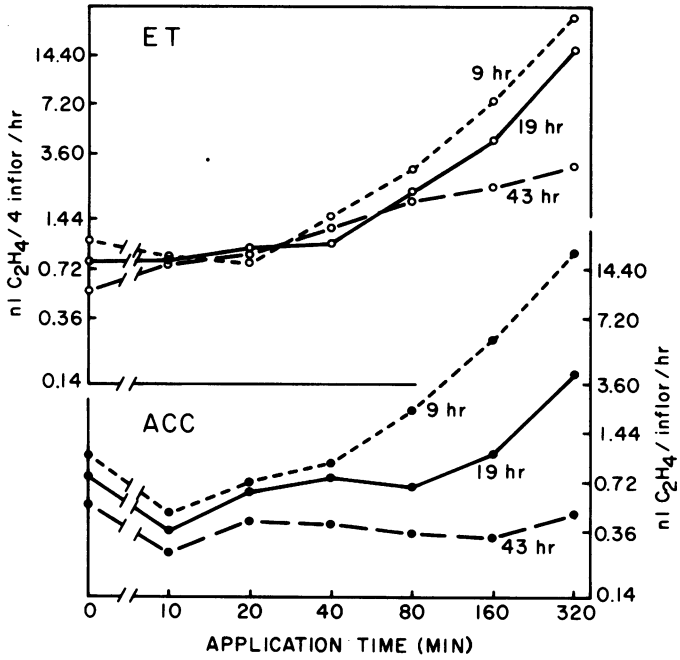


FIG. 4. The effect of ACC and ET on ethylene evolution from young inflorescences of excised "Manzanillo" olive shoots. Ethylene was determined 9, 19, and 43 h after application time. Chemical concentration $2 \times 10^{-3}$ M, four inflorescences per determination. (Mean inflorescence length 2.8 cm, mean fresh weight 50 mg.)

The movement of ACC in the transpiration stream was measured by ethylene evolution from mature leaves along the ACC-fed shoots at various times. At $2 \times 10^{-3}$ M ACC, ethylene evolution from leaves above the 7th segment was not changed by the treatments until 120 min of feeding. When $10^{-2}$ M ACC was used some increase in ethylene evolution was observed after 120 min even from leaves at the 13th node. The rate of ACC-stimulated ethylene evolution from leaves along the shoots was correlated with ACC concentration and the duration of feeding. The rate of ethylene evolution at each leaf level increased parallel to the increase in the application period. When longer shoots were used and cut off above the 13th node, the pattern of ethylene evolution from the leaves was similar but the amount smaller. In late April, reproductive shoots with developing inflorescences were exposed to ACC and ET. Ethylene evolution from the inflorescences increased due to both ACC and ET feeding treatments (Fig. 4). Ethylene evolution subsided more rapidly after ACC treatment than after the ET treatment, similar to the results shown earlier for the leaves.

Table III. *Effect of ACC and ET on Leaf Abscission of Vegetative and Reproductive "Manzanillo" Olive Shoots*[a]

Inflorescences were 2 cm long.

| Treatment $2 \times 10^{-3}$ M, 1 h | Time After Transfer to Water | | | Abscission Total |
|---|---|---|---|---|
| | 50 h | 80 h | 110 h | |
| | | | | % |
| Control | | | | |
| Vegetative | 0 | 0 | 0 | 0 |
| Reproductive | 0 | 0 | 0[a] | 0 |
| ACC | | | | |
| Vegetative | 0 | 0 | 0 | 0 |
| Reproductive | 0[a] | 0[b] | 0[b] | 0 |
| ET | | | | |
| Vegetative | 0 | 30 | 37 | 67 |
| Reproductive | 6 | 90 | 2 | 98 |

[a] Light chlorosis.
[b] Chlorosis.

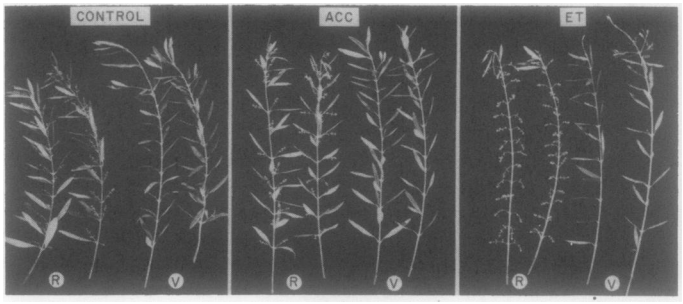


FIG. 5. The effect of ACC and ET on organ abscission of vegetative (V) and reproductive (R) "Manzanillo" olive shoots *in vitro*. (Chemical concentration $2 \times 10^{-3}$ M. Applied for 1 h. Recorded 60 h after transfer to water.)

Table IV. *Effect of ACC and Two Ethylene-Releasing Compounds on Ethylene Release and Abscission of Leaves from Excised Vegetative and Reproductive "Manzanillo" Olive Shoots 65 h After Treatment*

Mean leaf fresh weight was 238 mg.

| Treatment $10^{-3}$ M 1 h | Ethylene Release | | Leaf Abscission | |
|---|---|---|---|---|
| | Vegetative | Reproductive | Vegetative | Reproductive |
| | *nl/10 leaves·h* | | % | |
| Control | 7.6 | 10.1 | 0 | — |
| AL | 11.5 | 29.7 | 30 | — |
| ET | 14.0 | 31.1 | 51 | — |
| ACC | 6.2 | 8.6 | 0 | — |
| MSE ± | 1.7 | 3.7 | 5 | — |

In most cases, the reproductive shoots developed chlorosis in our *in vitro* system. The time of chlorosis appearance was dependent on the stage of inflorescence development. At early stages, when inflorescences were small, chlorosis appeared about 120 h after the excision. Later, when the inflorescences reach their full size, chlorosis appeared much sooner after about 24 h. ACC caused an enhancement in the onset of chlorosis on shoots with young inflorescences while ET prevented its occurrence (Table III). At later stages of inflorescence development, ET did not affect the appearance of chlorosis. Abscising leaves appeared normal in color and did not develop chlorosis. Limitation of light had no effect on either abscission or chlorosis in our system;

however, the laboratory lighting was many-fold less than full sunlight. Keeping the shoots in darkness during the experiment resulted in the same abscission results as those under laboratory light. Leaves on reproductive shoots are more sensitive to ET-induced abscission than those on vegetation shoots. Sixty min of $2 \times 10^{-3}$ M ET was enough to induce 90% leaf abscission 60 h thereafter on reproductive shoots while only 15 to 20% leaf abscission occurred under the same conditions on vegetative shoots (Fig. 5). ACC did not induce abscission in this experiment and no inflorescence abscission occurred in any of the treatments. Similar results were obtained when two different synthetic ethylene releasing compounds (AL, ET) were fed and compared to ACC. Sixty-five h after application, ethylene evolution from leaves on shoots fed with either of the substances was greater than the water-fed control or ACC treatment (Table IV). Although differences in induced leaf abscission between the compounds were found, all of them induced leaf abscission while no leaf abscission occurred on the ACC-treated or control shoots. In experiments with reproductive shoots with developing inflorescences just prior to anthesis, leaf chlorosis and drying occurred and no leaf abscission was found in either treatment or the control. Ethylene evolution from these leaves was generally greater than controls and treatment differences were still apparent.

## DISCUSSION

Olive leaves and inflorescences utilized ACC to evolve ethylene as do many other tissues (10). Ethylene evolution from olive leaves was rapid during and shortly after feeding ACC to excised shoots. Two to 3 h after feeding, the rate of ethylene evolution returned to the control level. Five other cyclopropanes fed to olive shoots did not result in marked increases in ethylene evolution. Although ACC was quickly converted to ethylene, it had little or no effect on endogenous ethylene evolution thereafter as the rate of ethylene evolution rapidly returned to control levels after ACC treatment. In contrast with ACC, ET increased the level of ethylene evolution from the leaves for an extended period of time (3–5 days). It is suggested that the rate of ethylene release after an ET feeding is dependent on catabolic capacity of the target tissue. This view is reinforced by the long duration of ethylene evolution from ET-fed stem tissues which may have a lower catabolic potential than the leaves. Our previous studies showed that ET does not enhance endogenous ethylene biosynthesis in the olive shoots as AVG (4, 22) had no effect on the rate of ET-dependent ethylene release. Although ET-induced leaf abscission is concentration-dependent (18, 19) large amounts of ethylene evolution for short periods of time are not enough to promote leaf abscission. Treating olive shoots with various concentrations of ethylene for different durations, Blumenfeld *et al.* (8) showed that a minimum application period of 10 h was needed to induce olive fruit abscission regardless of concentration. This also seems to be true for olive leaves. Furthermore, it had been shown by various workers (2, 7) that the breaking strength of an abscission zone in intact plants returns to that of the control when the ethylene source is removed before abscission is completed. Thus, the lack of abscission due to ACC feeding, in spite of the high ethylene evolution, could be explained on the basis of a rapid degradation of the precursor resulting in insufficient induction time. These results do not exclude the involvement of ACC in natural abscission as long as its supply to the target tissue is continuous and prevails for a long period. The lack of leaf abscission in some cases after ET feeding to reproductive shoots in our *in vitro* system could be due to rapid drying of the leaves on these shoots. This rapid drying could prevent the onset of the metabolic sequences leading to abscission.

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