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Ethylene Forming Activity from ACC in Citrus Leaf Discs: Influence of Light and Darkness

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Abstract

The influence of light and darkness incubation on ethylene forming activity from 1-aminocyclopropane-1-carboxylic acid (ACC) in citrus (*Citrus sinensis* L. Osbeck cv. 'Salustiana) mature leaf discs was studied. Leaf discs incubated 48 hours inlight produced 20 times greater ethylene than in darkness. Twenty-four hours light and darkness alternative incubations were carried out. In any case, transference of discs from the light to the dark resulted in inhibition of ethylene forming activity. Effects of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea, inhibitor of photosynthetic electron flow) and KCN (inhibitor of cytochrome oxidase) were studied,, DCMU at 0.1 mM concentration inhibited ethylene forming activity after 48 h incubation in light at 95%.However , ethylene forming activity was not affected by DCMU in the dark. On the other hand, 1 mMKCN stimulated considerably ethylene forming activity both in the light and dark. Incubation in a CO_2 enriched atmosphere did not affect ethylene forming activity in light.Therefore, respiratory CO_2 release could not be the responsible of ethylene forming activity inhibition in the dark. Increase on ethylene production in light from ACC in mature leaf discs is related with the ethylene forming enzyme (EFE) because of CO_2 $^+$ ion (inhibitor of EFE activity) reduced highly ethylene production from ACC both in the light and dark. Likewise mannitol (stimulator of EFE activity and ACC synthesis) enhanced ethylene production from ACC both in the light and in the dark. Cycloheximide (inhibitor of protein synthesis) also inhibited ethylene production from ACC. Therefore, enzyme synthesis could be required for the ethylene forming activity from ACC.

Abbreviations: ACC: 1-aminocyclopropane-1-carboxylic acid, CH: cycloheximide, DCMU: (3–(3,4-dichlorophenyl)-1,1-dimethylurea, EFE: ethylene forming enzyme.

Introduction

Gepstein and Thimann (3) reported that light inhibited ACC-dependent ethylene production. Subsequently some investigators have observed that light markedly inhibited ethylene production in various green leaf tissues at the step of ACC conversion to ethylene (2,4,9). In these studies CO_2 levels into the incubation flasks were not maintained, and may oscillate as the result of photosynthetic or respiratory activities. Grodzinski et al. (5) and Kao and Yang (6) showed that light inhibitory effect on the ethylene production from ACC was restored by an external source of CO_2 to a level approaching or exceeding to level observed in the dark. Moreover, CO_2 did not stimulated ethylene production in the dark. These autors concluded that inhibition of ethylene production by light might result from a decrease in CO_2 concentration into the incubation flasks.

In the present work, we have studied the influence of light and darkness on ethylene forming activity from ACC in citrus leaf discs, and its relation with CO_2 levels into the flasks.

Materials and Methods

Plant material and treatments

Eight to 12-month-old leaves were harvested from mature citrus (*Citrus sinensis* (L.) Osbeck cv. 'Salustiana') trees grown in Montcada (Valencia). Discs, 12mm in diameter, were excised with a cork borer and kept in a moistened Petri dish until treatment. Except when otherwise noted, 10 discs, weighing about 0.30 g. were placed in a 27 ml erlenmeyer flask with 1 ml of 1 mM ACC and sealed with a rubber serum cap. Incubations were carried out in a culture chamber at 25°C. Flasks incubated in light were illuminated continuously with 5 fluorescent tubes Phillips TLD 18 W (light intensity 95 $\mu E.m^{-2}.s^{-1}$). Flasks incubated in the dark were wrapped in aluminium foil and placed in the dark.

Chemicals

ACC, DCMU, cycloheximide were purchased from Sigma Chemical Co. All the other reagents were of analytical grade purity. Deionized distilled water was used in all experiments.

Determination of ethylene

At the end of each incubation 1 ml gas sample was taken from the incubation flasks and injected in a gas chromatograph equipped with an activated alumina

column and a flame ionization detector. Injections of known ethylene standards were used to calibrate retention times and peak heights for the gas samples.

Determination of ACC

Ten discs, weighing about 0.3 g. were ground with a mortar and pestle in 5 ml 5% sulfosalicylic acid, and the extract was centrifuged for 20 min at 5.000 g. ACC in the supernatant was assayed by the method of Lizada and Yang (7).

Results

Effects of light and dark treatments on ethylene production from 1 mM ACC in citrus leaf discs

Discs incubated continuously in the dark produced very little ethylene from 1 mM ACC. When the incubations were carried out in the light, ethylene production increased markedly (Fig. 1) At 48 h in the light maximal values on ethylene production were observed. At this moment leaf discs produced in light 20 times greater ethylene than in darkness.

In order to ensure that this is a effect of light and dark treatments, and it is not due to a different absortion of ACC from the incubation medium, concentration of ACC into the tissues and ethylene production after 24 h on ACC 1mM were determined both in light and dark. Table 1 shows the results obtained. Thus, whereas ACC concentrations were the same both in light and dark, ethylene production in light is approximately 4 fold higher than in the dark.

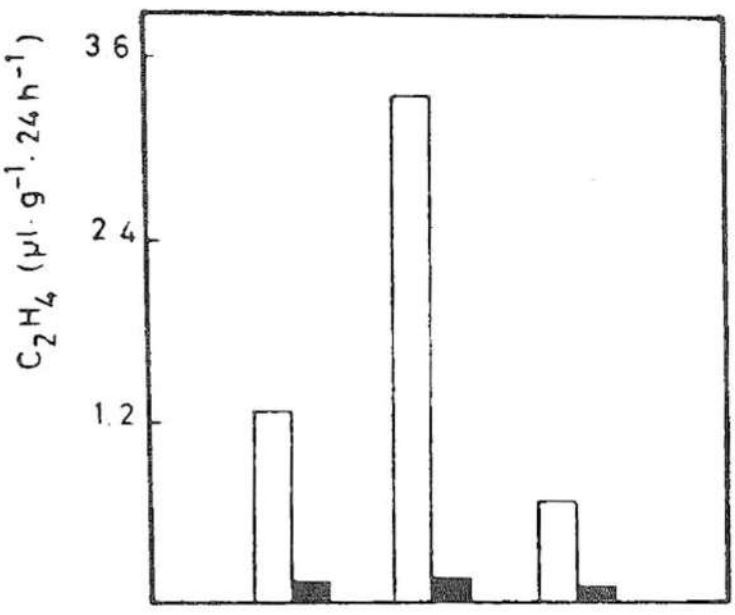

Figure 1: Ethylene production from 1 mM ACC in citrus leaf discs incubated in 27 ml sealed flasks. Discs were incubated for 24 h periods in light □ or darkness ■. At the end of each 24 h period 1 ml gas sample was withdrawn with a syringe and assayed by gas chromatography. After sampling flasks were flushed with a air stream and stoppered with a rubber serum cap.

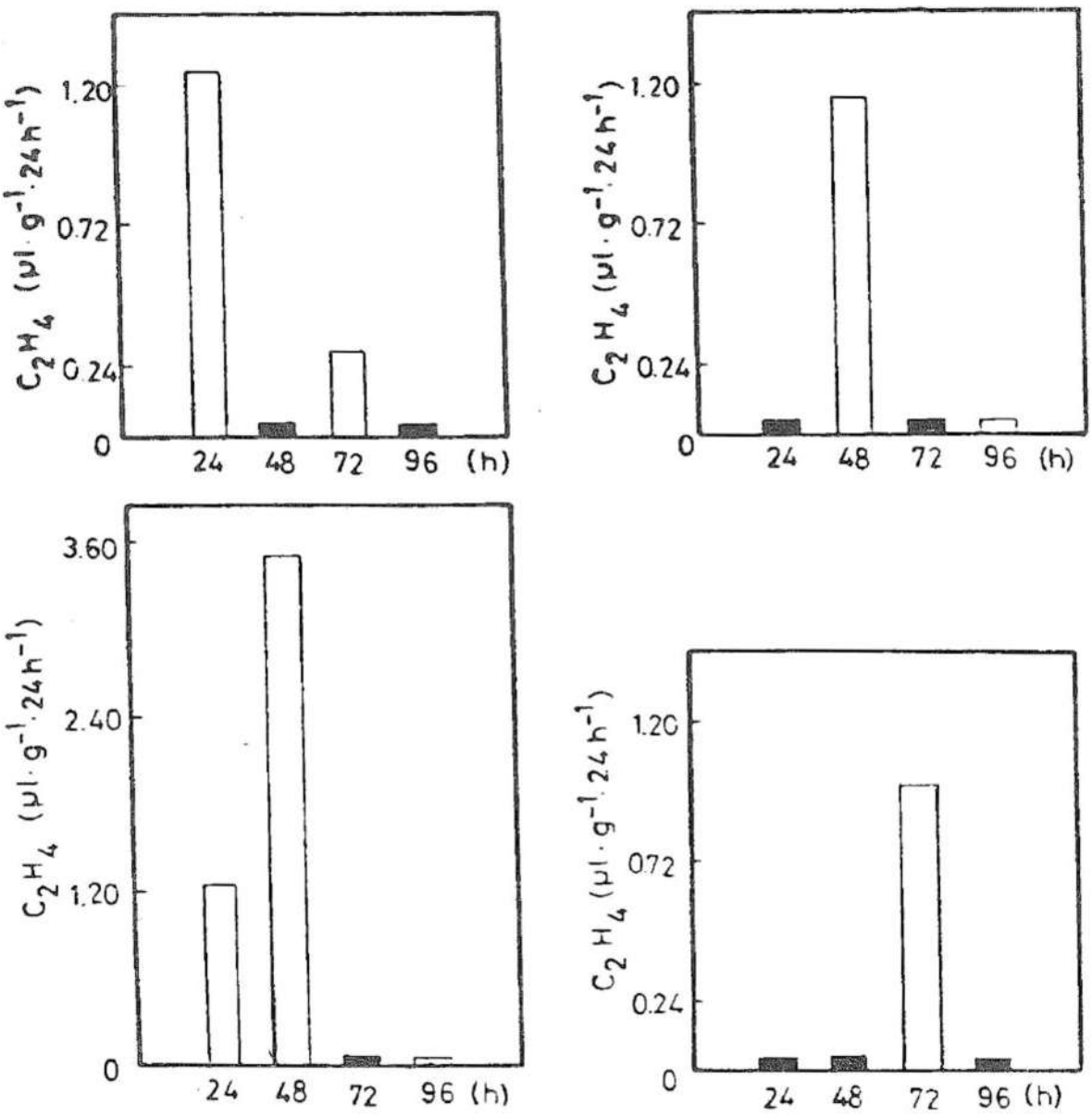

Figure 2: Ethylene production form 1mM ACC in citrus leaf discs incubated for 24 h periods in light or darkness. After each 24 h period 1 ml gas sample was collected and assayed by gas chromatography. After sampling flasks were flushed with a air stream, stoppered with a rubber serum cap and transferred to the incubation conditions (light or darkness) indicates in the figure.

When 24 h light and darkness alternative incubations were carried out, we have observed that, in any case,transference of discs from the light to the dark resulted in inhibition of ethylene forming activity (Fig. 2). After a 24 h period in the dark ethylene forming activity was restored in the light, and reached production levels like discs incubated 24 h in the light. After 48 h in the dark, ethylene forming activity from 1 mM ACC was also restored, but at lower levels than under 24 h darkness — 24 h light period. A 24 h light-24 h darkness period decreased significantly ethylene production form ACC in a subsequent 24 h light period in relation to discs incubated

48 h in the dark. It could be due to progressive tissue degradation during incubation. The effect of ethylene on tissues senescence is well known, and ethylene released from light treated discs would acelerate tissue degradation.

Table 1: ACC concentration and ethylene production in citrus leaf discs after 24 h incubation on 1 mM ACC in light or darkness. At the end of incubation discs were washed, frozen with liquid N_2 and assayed for ACC or enclosed into 14 ml sealed tubes for 1 h for ethylene production measurement.

	(ACC) $\mu mol.g^{-1}$ (fresh weight)	C_2H_4 $nl.g^{-1}.h^{-1}$
Light	3.170±0.443	274.11±32.24
Darkness	3.121±0.423	74.70±3.65

In the experiment reported in Fig. 3 leaf discs were incubated on 1mM ACC solution in a CO_2 enriched atmosphere (10%) or in a free CO_2 atmosphere, where CO_2 was removed with a KOH trap. CO_2 did not affect ethylene production both in the light and dark.

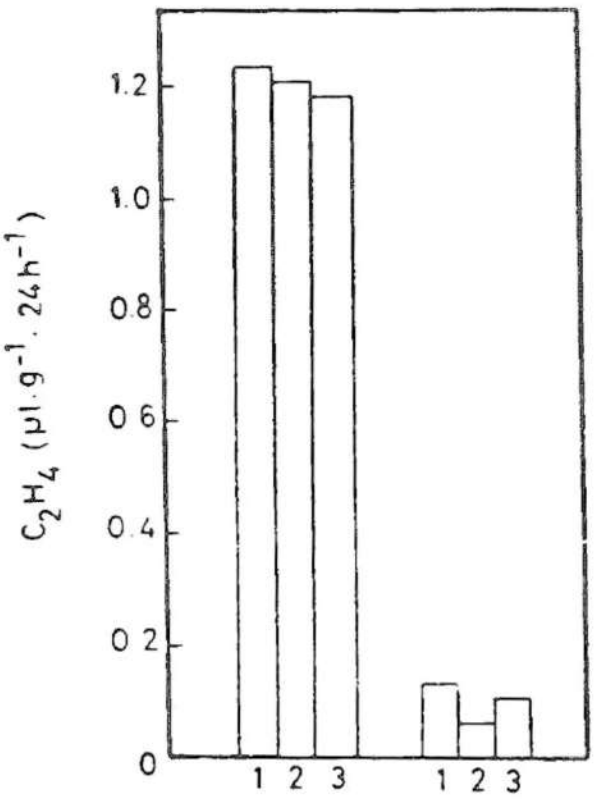

Figure 3: Effect of CO_2 on ethylene production form 1 mM ACC in citrus leaf discs in light and dark. Discs were incubated for 24 h in 27 ml sealed flasks: 1) with 1 ml 1 mM ACC, 2) with 1 ml 1 mM ACC with a KOH trap for CO_2, 3) with 1 ml 1 mM ACC in a CO_2 enriched atmosphere (10% CO_2) by injecting CO_2 into the incubation flasks.

Fig. 4 shows the effect on ethylene production from 1 mM ACC in citrus leaf discs when DCMU (inhibitor of photosynthetic electron flow) was added to the incubation medium. C_2H_4 production was inhibited about 95% in the light by DCMU whereas no effects were detected in the dark. KCN (inhibitor of cytochrome oxidase) enhanced ethylene production from ACC both in light and dark.

Ethylene production from ACC was increased by 100 mM mannitol both in the light and in the dark (Fig. 5), whereas Co^{2+} showed an inhibitory effect (Fig. 6).

Fig. 7 shows the effects of cycloheximide (CH) treatments on ethylene production from 1 mM ACC. CH (10–6M) inhibited ethylene production both in light and dark, but this inhibition is higher in the light. Discs incubated in the light with CH produced ethylene levels like discs incubated in dark without CH.

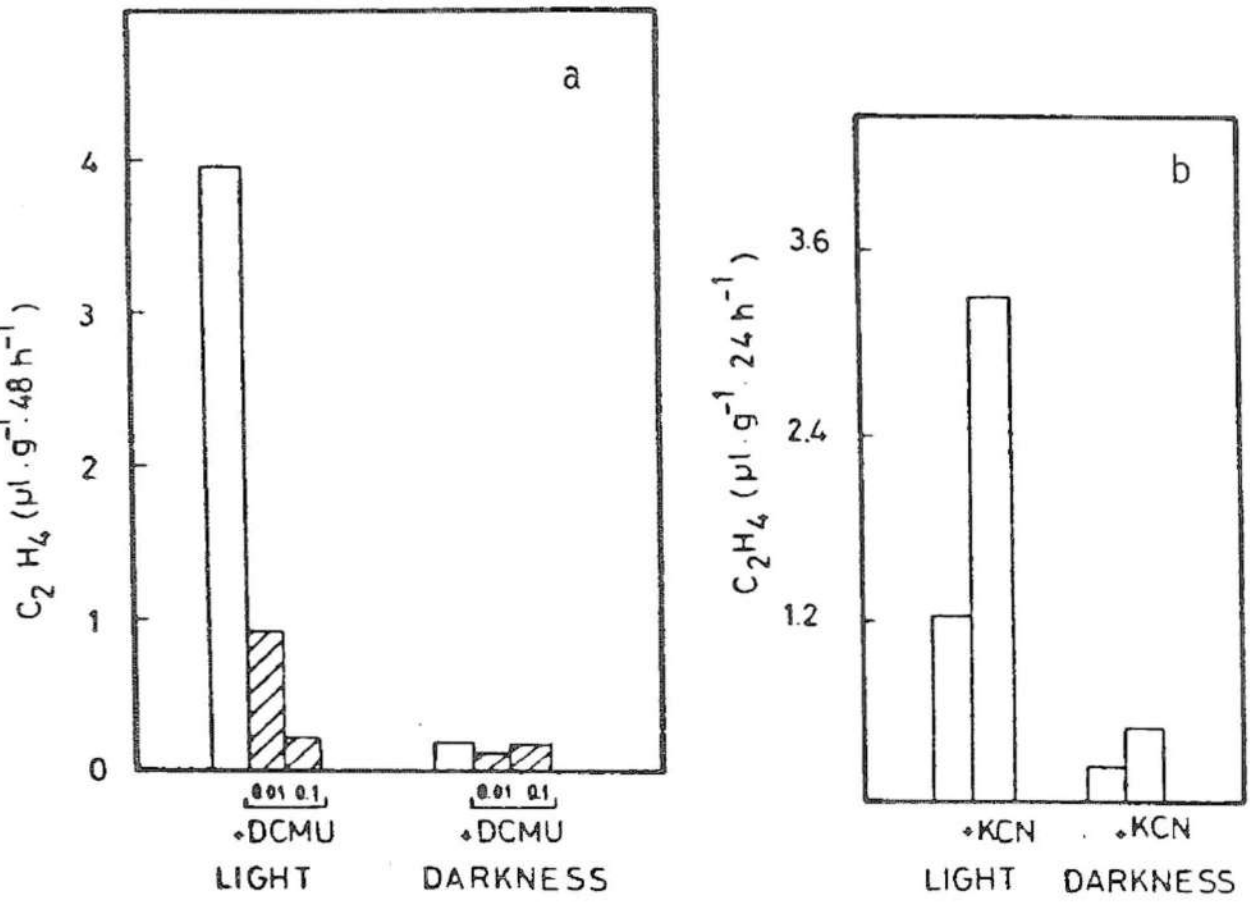

Figure 4. Effect of DCMU and KCN onethylene production from 1 mM ACC in citrus leaf discs. a) Discs were incubated for 48 h in a solution containing 1 mM ACC or 1 mM ACC + DMCU at two different concentrations (0.01 and 0.1 mM). b) Discs were incubated for 24 h in a solution containing 1 mM ACC or 1 mM ACC + 1mM KCN.

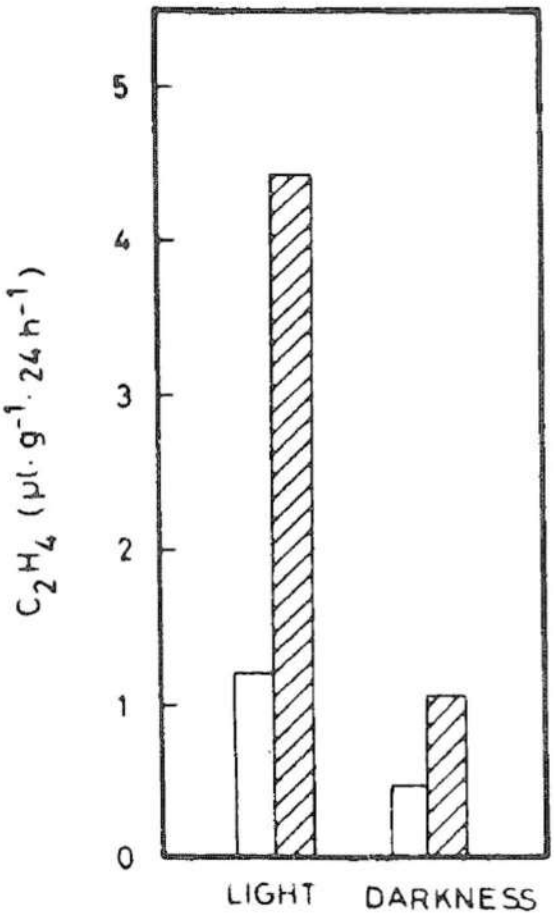

Figure 5: Effect of mannitol on ethylene production from 1 mM ACC in citrus leaf discs. Discs were incubated for 24 h in a solution containing 1 mM ACC or 1 mM ACC + 100mM mannitol. At the end of incubation ethylene was assayed by gas chromatography.

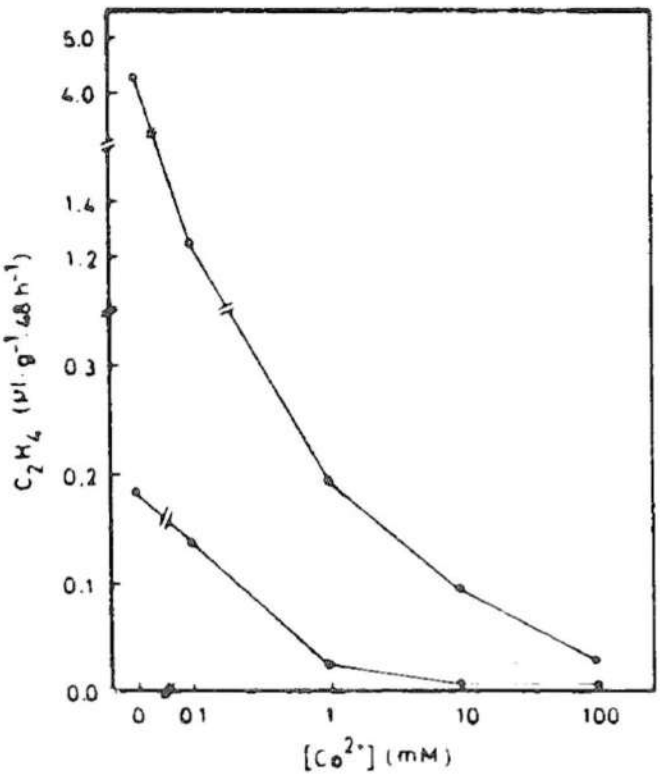

Figure 6: Ethylene production form 1 mM ACC in citrus leaf discs incubated in 27 ml sealed flasks with 1 mM + different concentrations of $CoCl_2$. Discs were incubated for 48 h in light (o) or darkness (o), and ethylene was assayed by gas chromatography.

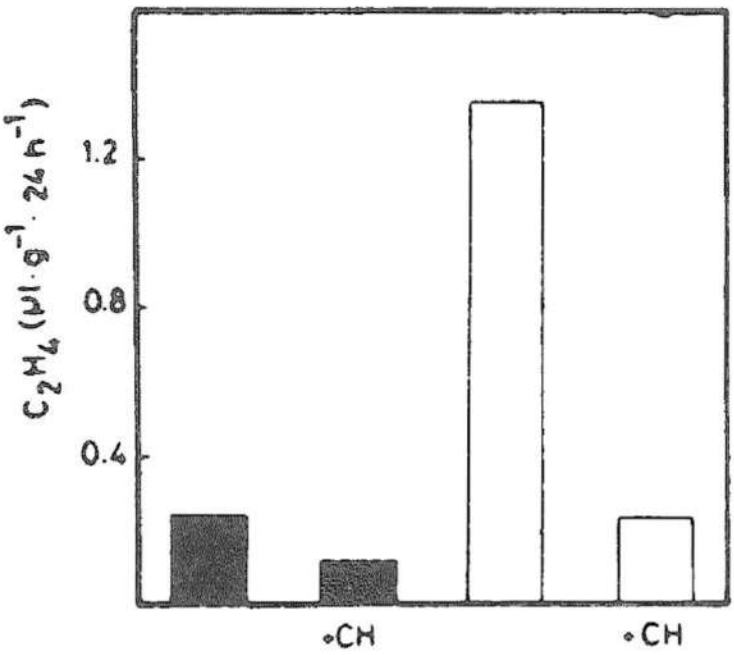

Figure 7: Influence of cycloheximide (CH) on ethylene production in citrus leaf discs. Discs were incubated for 24 h in light or darkness in 1 ml 1 mM ACC with or without 10^{-6}M cycloheximide. At the end of each incubation ethylene was assayed by gas chromatography.

Discussion

Our results showed that in citrus leaf discs ethylene production from ACC is markedly stimulated in the light, and this effect was not affected by the CO_2 levels in the incubation flasks. Light stimulatory effect on ACC-dependent ethylene production was according to precedent works (5,6). These authors reported that, light treated tissues released lower amounts of ethylene than dark treated tissues, when CO_2 levels into the flasks were not maintained. However, an external source of CO_2 stimulated ACC-dependent ethylene production in light. Citrus leaf discs not showed CO_2-dependent ethylene production from ACC, which it seems not consistent with results obtained in other species, where CO_2 levels into the incubation flasks are a key factor regulating ethylene release from ACC in leaf tissues.

Ethylene production from ACC in citrus leaf discs exposed to light was inhibited by DCMU, an inhibitor of photosythetic electron transport. Gepstein and Thimann (3) and De Laat et al., (2) observed the DCMU counteracts the inhibitory effect of light on ethylene production from ACC and concluded that light inhibition was related to the photosynthetic system. In these experiments, the inhibition of photosynthesis by DCMU probably reduced CO_2 uptake by leaf tissues, and thus CO_2 into the incubation flasks was not depleted. In our experiment the inhibitory effect of DCMU on ethylene production from ACC, was independent of the CO_2 levels in the incubation flasks. This effect could be interpreted as ACC-dependent ethylene production requires photosynthetic rich-energy compounds.

Surprisingly, KCN enhanced ACC-dependent ethylene production both in light and dark. More research is needed to explain this effect. In this way, plant mitochondria have a cyanide-resistant respiratory chain, which may be stimulated when KCN was added to incubation medium (8), and some authors (8) reported a realtionship between ethylene and cyanide-resistant respiration in some climateric fruit tissues.

Light stimulating effect on ACC-dependent ethylene production appears to be mediated by the regulation of the ethylene forming enzyme (EFE) at the level of synthesis or activation as proposed by De Laat et al., (2). Mannitol, which enhances EFE activity, and CO^{2+}, a known specific inhibitor of EFE, increased and decreased respectively the ACC-dependent ethylene production in citrus leaf discs. Moreover, cycloheximide, that inhibits protein synthesis, dramatically reduced ethylene production from ACC, indicating that enzyme synthesis could be required.

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