Does Light Inhibit Ethylene Production in Leaves?1

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ABSTRACT

The effect of light on the rate of ethylene production was monitored using two different techniques—leaf segments incubated in closed flasks versus intact plants in a flow-through open system. Three different plants were used, viz sunflower (Helianthus annuus), tomato (Lycopersicon esculentum), and soybean (Glycine max). Experiments were conducted both in the presence and absence of 1-aminocyclopropane-1-carboxylic acid (ACC).

The results obtained indicate that, in all three species studied, light strongly inhibits ethylene production when cut leaf segments are incubated in the presence of ACC in closed flasks. When ethylene measurements are made with ACC-sprayed intact plants using a continuous flow system, the effect of light on ethylene production is only marginal. In leaf segments of sunflower and soybean incubated only in distilled H2O in closed flasks, light promotes ethylene production. In tomato, there is no difference between the rate of ethylene production between light and darkness under such flasks. When measurements are made with intact plants in a continuous flow system, the rate of ethylene production is almost identical in light and darkness, in the three plants studied.

It is concluded that the effect of light on cut leaf segments incubated in the presence of ACC in closed flasks can be attributed to the techniques used for these measurements. Light has little effect on ethylene production by intact plants in an open system.

There have been a number of reports that suggest that white light inhibits ethylene production (3, 6–9). This inhibitory effect of light was first reported by Gepstein and Thimann (6) in tobacco and oat leaf segments. These workers used ACC2 in the incubation medium to increase the basal rate of ethylene production. De Laat et al. (3) reported that endogenous ethylene production of tobacco leaf discs was similar in light and darkness but the conversion of ACC to ethylene was reversibly inhibited by light. Grodzinski et al. (7) have recently published a paper indicating that light inhibits ethylene production from Xanthium leaf discs both in the presence and absence of ACC, although the effect was much more pronounced in the presence of ACC. Almost simultaneously with the report of Grodzinski et al. (7), Kao and Yang (8) have reported that light inhibits ethylene production in rice leaf segments, but the effect is more pronounced in the absence of ACC.

In all these cases, excised leaf segments were incubated in closed flasks and the ethylene samples were withdrawn after varying periods of time. Wright (9) reported that light inhibited ethylene production induced by water stress in both excised wheat leaf segments and intact seedlings. Light also inhibited ethylene production in nonstressed leaf segments but the effect was much less pronounced. No data were reported for the effect of light on ethylene production in intact nonstressed seedlings. For all Wright's experiments, the tissues were kept in closed systems and ethylene was determined by head space analysis.

We have recently developed techniques in our laboratory that allow the measurement of rate of ethylene production in intact plants using a continuous flow system (1, 5). Using these methods, we found that in intact sunflower plants, light had no inhibitory effect on the rate of ethylene production (2). If anything, there was a slight promotion in the rate of ethylene production after the plants were transferred from darkness to light but the rate of ethylene production quickly subsided to a level comparable to that obtained in darkness.

The discrepant results from various laboratories were puzzling. The present paper assesses how much of the variation can be attributed to the different experimental techniques employed.

MATERIALS AND METHODS

Sunflower, tomato, and bean plants were grown in continuous light in a growth chamber maintained at 26 ± 1°C and RH of 75%. The intensity of light (400–725 nm) was constant at 125 µE m−2 s−1.

Procedures for measurement of ethylene production from intact plants were the same as described previously (4). Dark treatment was given by wrapping the whole apparatus with a thick, black plastic polyethylene bag. The temperature in the cuvette was kept constant at 26°C by circulating water in the water-jacket of the cuvette, from a constant temperature water-bath. In intact plants, ACC treatment was given by spraying both sides of the leaves to the dripping stage with an atomizer. The plant was immediately set in the cuvette and the flow of purified air was started through the cuvette. The first ethylene measurement was made after waiting for an additional 1.5 h. In plants sprayed with ACC, ethylene collections for only 5 min were sufficient to allow accurate quantitative measurements.

For the experiments on excised segments, tissues of second leaf pairs from 3-week-old plants were excised in exactly the same way for all treatments. In tomato plants, the opposite pinnae were used in dark versus light treatments to prevent any variability because of the physiological age of the tissue. In sunflower and bean plants, each leaf was cut along the midrib and the two halves from the same leaf used for dark and light incubation, respectively. The leaf segments were floated on either 5 ml of distilled H2O or ACC in a 50-ml flask flushed with air. The flasks were then sealed with serum stoppers and incubated either in light or darkness for different durations of time. Direct exposure of the air in the flask with the serum stopper was prevented by inserting a layer of aluminum foil between the flask and the stopper. Ethylene determinations were made by withdrawing 5 ml of air sample from the head spaces and analyzing it on a gas chromatograph (Hewlett Parkard 5880) equipped with a Porapak Q column maintained at 40°C (1).

ACC was obtained from Calbiochem-Behring Corporation. In
all cases, the concentration of ACC solution used was 1 mm. Control experiments were conducted to test the stability of ACC in solutions exposed to light; no detectable amounts of ethylene were found after 16 h of incubation.

All experiments were repeated at least once with similar results. The data for only one set of representative experiments have been presented here. The fresh weight equivalents of unit dry weight for sunflower, soybean, and tomato were 7.3, 6.2, and 7.5, respectively.

**RESULTS**

We have done our experiments with three plants: sunflower (*Helianthus annuus* L.), soybean (*Glycine max*), and tomato (*Lycopersicon esculentum*).

Sunflower. When excised leaf segments of sunflower were incubated in 1 mm ACC in closed flasks, the rate of ethylene production in the light was much less than that in the dark (Table I). However, in the absence of exogenous ACC, the leaf segments produced more ethylene in light than in darkness. When intact shoots of sunflower were sprayed with ACC and the rate of ethylene production was measured in a continuous flow system, there was only a slight and transient increase in the rate of ethylene production when the seedlings were transferred from light to darkness (Fig. 1A). For ease of comparison with the data from cut segments in closed systems, we took the average of three sequential readings taken in light and in darkness. The average rate of ethylene production in light was 149.3 nl g⁻¹ dry wt h⁻¹ as compared to 163.7 in darkness. We have already reported that the rate of ethylene production was not significantly affected in intact plants when the plants were transferred from light to darkness in the absence of ACC (2). When the experiments were repeated using the present experimental design, the results obtained were very similar (Table II).

Preliminary experiments indicated that when the plants were initially exposed to dark followed by light, the pattern of change in ethylene production was identical to that obtained with shifts from light to darkness. In all subsequent experiments, the plants were first exposed to light followed by darkness.

Soybean. Light inhibited the rate of ethylene production in the excised leaf segments incubated in ACC in closed flasks (Table I), as it did in sunflower. In the leaf segments floated on water, light again promoted the rate of ethylene production.

In intact plants sprayed with ACC, there was a gradual increase in the rate of ethylene production in light until the rate stabilized around 350 nl g⁻¹ dry wt h⁻¹ (Fig. 1B). This initial increase may represent a slow rate of uptake of ACC through the relatively thick cuticles of soybean leaves. When the plants were transferred from light to darkness, a slight increase in the rate of ethylene production was observed. However, the percent increase was only about 30% as compared to 225% observed in excised leaf segments incubated in ACC in closed flasks. When measurements were made in the absence of ACC in intact plants, there was no difference between the rate of ethylene production in light or darkness (Table II).

Tomato. Light inhibited ethylene production in leaf segments incubated in ACC was observed in this plant as well (Table I). In fact in some experiments, the rate of ethylene production in cut leaf segments incubated in ACC was 10 times as much in darkness as compared to that observed in light. However, in leaf

### Table I. Comparison of Rate of Ethylene Production in Excised Leaf Segments of Plants Incubated in Light or Darkness, in the Presence and Absence of ACC

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>ACC Ethylene Production after Following Treatments</th>
<th>Water Ethylene Production after Following Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
<td>Dark</td>
</tr>
<tr>
<td>Sunflower</td>
<td>213.0</td>
<td>1090.2</td>
</tr>
<tr>
<td>Soybean</td>
<td>215.1</td>
<td>693.2</td>
</tr>
<tr>
<td>Tomato</td>
<td>565.1</td>
<td>2062.7</td>
</tr>
</tbody>
</table>

**FIG. 1.** Effects of light and darkness on the rates of ethylene production by intact ACC-sprayed plants in a continuous flow open system. Readings at time 0 represent the first reading taken in the light after the plant had equilibrated. The position of the arrow indicates the start of the dark treatment. Sunflower (A), Soybean (B), Tomato (C).

**Table II. Comparison of Rate of Ethylene Production in Intact Plants of Sunflower, Soybean and Tomato in Light and Darkness, in the Absence of ACC**

The rates were measured using a continuous flow system. The basic experimental design was the same as in Figure 1. The data are the average rates of ethylene production determined in three sequential readings in the light and in darkness.

<table>
<thead>
<tr>
<th>Species</th>
<th>Rate of Ethylene Production</th>
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<tbody>
<tr>
<td></td>
<td>Light</td>
</tr>
<tr>
<td>Sunflower</td>
<td>20.4</td>
</tr>
<tr>
<td>Soybean</td>
<td>6.4</td>
</tr>
<tr>
<td>Tomato</td>
<td>14.7</td>
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</tbody>
</table>
segments incubated in water, there was no significant difference between the rate of ethylene production in light and darkness.

There was virtually no difference in the rate of ethylene production in intact plants sprayed with ACC between light and darkness (Fig. 1C). The average rate of ethylene production from these sequential determinations in light and darkness was 485.9 and 479.3 nl g⁻¹ dry wt h⁻¹, respectively. Similar results were obtained in the absence of ACC (Table II).

In all three plants tested in this investigation, the rate of ethylene production increased rapidly by several-fold when the plants were transferred from light to darkness (Fig. 1A). This was true for the controls. Another point that is obvious from these data is that the rate of ethylene production in darkness in cut leaf segments of sunflower and tomato incubated in ACC in closed flasks was always 3 to 4 times more that observed in intact plants sprayed with ACC in flow-through systems. In soybean plants, the difference was not as significant. In the absence of ACC, this difference was most pronounced in tomato plants. These differences may be a manifestation of the relative degree of the well-known 'wounding' effect in excised leaf segments.

**DISCUSSION**

The data presented in this paper show that in excised leaf segments of sunflower, tomato, and soybean plants incubated in closed systems in the presence of ACC, light strongly inhibits the rate of ethylene production. These findings are in accord with the results obtained by earlier workers (3, 6–8). However, when the rate of ethylene production is monitored in intact shoots of the same plants using continuous flow systems, a completely different picture emerges. In intact plants, there is a marked effect of light on ethylene production by ACC-treated plants. Its significance in comparison with the effect observed with excised plants incubated in closed systems.

In leaf segments of sunflower plants in closed systems, light consistently promoted the rate of ethylene production when the leaf tissue was incubated in distilled water. In leaf segments of soybean, light similarly promoted the rate of ethylene production but not to the same degree as in sunflower. In tomatoes, there was no effect of light on the rate of ethylene production under identical conditions. These data are in marked contrast to those reported by earlier workers (7, 8) with other plant materials. One possible explanation is that in leaf segments of these plants incubated in water, the level of free ACC may be limiting. No effect was observed when plants were transferred from light to darkness in the absence of ACC, in a continuous flow system.

Grodzinski et al. (7) and Kao and Yang (8) reported that increasing the CO₂ concentrations during the light period alleviated the inhibitory effect of light. This is not surprising. In our earlier papers, we have demonstrated that CO₂ promotes the rate of ethylene production (4) and that the effect could be observed only in the presence of light (2). The effect of increased CO₂ concentration was observed under those conditions where there was no significant effect of light itself on rate of ethylene production as compared to darkness (2).

Kao and Yang (8) have attempted to explain the inhibitory effect of light on the basis of changes in CO₂ concentration in the incubation flasks. However, their experiments were done using leaves discs in closed systems. In a closed system (e.g. 7 and 8) where CO₂ concentration is continually changing, the effect of changes in CO₂ concentrations cannot be adequately assessed.

In our experiments with closed systems reported in this paper, more CO₂ accumulated in darkness than in light. As expected, the accumulation was dependent on such factors as the duration of incubation and amount of tissue. There was no significant effect of ACC on the accumulation of carbon dioxide in the flasks. We have already pointed out a number of other potential pitfalls of incubating excised plant tissues in closed systems for measurements of rates of ethylene production (2, 5). For instance, a potential autocatalytic or autoinhibitory effect of ethylene, particularly in the presence of ACC when there is so much ethylene generated, must be considered, in addition to the wounding effect.

We have already reported that there is a considerable variability in rates of ethylene production among different plants of the same species and age (2, 4), when measured in a continuous flow system. The problem is further compounded by possible differences in ACC deposition and uptake under different experimental conditions. In closed systems, we were limited in the number of replicates we could use for each treatment because changes in gaseous composition around the tissue are a function of time of incubation and that could potentially affect the data among replicates. Therefore, the data for only one set of replicates has been presented here. However, for a given experimental design, the same general trend was consistently obtained in all experiments.

We are testing the validity of the hypothesis based on photo-synthetic effect of light, in intact plants using continuous flow systems, and the data will be published elsewhere. In this paper, we wish to emphasize the dangers inherent in certain commonly used techniques for ethylene measurement. Both wounding and the use of a closed system undoubtedly contribute to the response observed. The results presented in this paper demonstrate that the inhibition of the rate of ethylene production in ACC-treated plant tissues in light as reported by several workers merely represents a manifestation of the experimental techniques employed. In fact, light has a very small effect on the rate of ethylene production when the measurements are made with intact plants treated with ACC in continuous flow systems. Further, in view of our results with plants in the absence of exogenous supply of ACC, the effect of light on ACC-treated plants cannot necessarily be extrapolated to represent a similar effect under natural conditions.

**LITERATURE CITED**

1. Bassi PK, MS Spencer 1979 A cuvette design for measurement of ethylene production and carbon dioxide exchange by intact shoots under controlled environmental conditions. Plant Physiol 64: 488–490