Does Ethylene Mediate Root Growth Inhibition by Indole-3-Acetic Acid?

W. A. Andreae\textsuperscript{1}, M. A. Venis, F. Jursic and T. Dumas

Research Institute, Canada Agriculture, University Sub. P. O., London, Ontario, Canada

Received May 13, 1968.

Abstract. The effects of ethylene and of indole-3-acetic acid (IAA) on growth of excised pea root sections have been compared under a variety of conditions. After 16 hours treatment the inhibitory action of IAA is fully reversible on transfer of the root sections to IAA-free solutions. In contrast, inhibition by ethylene is almost totally irreversible. IAA inhibits growth from zero time; ethylene is generally without effect during the first 3 to 6 hours. The inhibitory action of ethylene is dependent on factors such as tissue age and solution composition which have no major effect on IAA inhibition. Ethylene production is enhanced by 100 \( \mu \text{M} \) IAA, but conditions which reduce the rate of ethylene evolution 2 to 3-fold at the same IAA concentration fail to affect the inhibitory action of IAA on elongation. It seems unlikely that ethylene can play more than a minor role in mediating inhibition of pea root growth by IAA.

It has been suggested (3) that the inhibition of pea root growth which occurs in response to applied indole-3-acetic acid (IAA) is due not to a direct action of IAA, but to auxin-induced ethylene formation. Previous detailed studies in this laboratory on root growth inhibition by IAA (2) led us to question this conclusion and to compare more closely the effects of IAA and of ethylene on growth of excised pea roots. While ethylene is certainly produced in response to IAA, the effects of applied ethylene and of IAA can be distinguished in so many ways that it seems doubtful whether the very small amounts of ethylene evolved can be of major significance in mediating the inhibitory action of IAA.

Materials and Methods

Pea seeds (\textit{Pisum sativum} L., var. Alaska) were allowed to swell in water for 6 hours (2-day and 3-day peas) or 16 hours (3 and one-half day peas), then germinated in vermiculite in a dark growth room at 24\(^\circ\). The roots averaged about 2.5 cm, 5 cm or 6 cm in length respectively at these different stages of germination, (48 hr, 72 hr, and 88 hr after initial soaking).

For growth studies, ten 5 mm root tips were shaken in a buffered medium in Erlenmeyer flasks (nominal volume 125 ml; actual volume ca. 160 ml) at room temperature (22-23\(^\circ\)) in the dark. Two media were used: Medium A, used in previous studies in this laboratory (2), consisted of 0.5\% sucrose (w/v), 1 \( \text{mm} \) calcium nitrate, 5 \( \text{mm} \) potassium phosphate, pH 6.3. Medium B, used by Chadwick and Burg (3), was 2\% sucrose (w/v), 5 \( \mu \text{M} \) cobaltous chloride, 5 \( \text{mm} \) potassium phosphate, pH 6.8. [Chadwick and Burg (3) reported their phosphate concentration as 50 \( \text{mm} \), but this was an error (Chadwick, personal commun.). Phosphate concentrations in excess of 10 \( \text{mm} \) greatly inhibit growth of pea roots.] Unless stated otherwise, 20 ml of medium A or 5 ml of medium B per flask were used, to correspond with previous practice in the respective laboratories. Changes in fresh weight and in length were determined at appropriate intervals. To study recovery from IAA inhibition, tissue was transferred after 16 hours either to fresh IAA or to buffer for 4 hours. Weight and length were again measured, the solutions renewed once more and measurements repeated after a further 4 hours. A similar protocol was followed for recovery from ethylene inhibition. Ethylene (99.5\% purity) was injected into flasks sealed with silicone rubber septa, using a gas-tight syringe.

Ethylene production was determined by gas chromatography using the Burrell K-7 instrument with flame ionization detector. The stainless steel column was packed with alumina 40 to 60 mesh, 8 feet (2.4 m) by one-eighth inch (3.175 mm) diameter, operated at 40\(^\circ\) with nitrogen carrier at 55 cm\(^3\) per minute. Amounts of ethylene as low as 0.1 to 0.2 ml in a 5 ml gas sample could be determined accurately. Levels of ethylene below 0.1 ml could be readily detected, but only an estimate of the amount could be made. In practice, such estimation was required only for the very low levels produced in the absence of IAA. Ethylene production was determined using 2-day peas and medium B. Each flask contained 20 root tips, weighing 90 to 100 mg.

All experimental figures are the means of duplicate determinations. Vertical bars through or adjacent to experimental points represent twice the sample standard deviation.

\textsuperscript{1} Deceased.
Results

Kinetics of Inhibition. Chadwick and Burg (3) claim that ethylene inhibits elongation from zero time, while fresh weight gain is not affected for the first 5 hours. They present no comparable data for IAA, but past experience in this laboratory (2) has been that the course of IAA inhibition of weight increase is nearly always essentially linear with time as long as the concentration of applied IAA is maintained fairly constant. Initially therefore, the kinetics of IAA- and ethylene-inhibited root growth were compared under the conditions used by Chadwick and Burg. While IAA inhibits both weight gain and elongation from zero time (fig 1), it is evident that 100 ppm ethylene is effective only after a lag of 3 hours. This concentration of ethylene was chosen for the present study because it lies well within the range, 10 to 1000 ppm, which was reported by Chadwick and Burg (3) to cause maximum inhibition of root growth.

Similar experiments were carried out using roots from 3-day (fig 2) and 3 and one-half day (fig 3) peas. The initial kinetics of ethylene action on the 3 and one-half day roots resemble more closely those reported by Chadwick and Burg (3) for 2-day roots, but are, nevertheless, clearly distinct from those of IAA inhibition. Again, while ethylene inhibits growth of 3-day roots from zero time (using medium B), inhibition becomes much greater after 6 hours, unlike the course of IAA-inhibited growth (fig 2). Comparison of figures 1, 2 and 3 demonstrates that the age of the tissue has a marked effect on the response to ethylene, but little or no effect on the course of IAA inhibition.

Reversibility of Inhibition. In the experiments described in figures 2 and 3, recovery from inhibition was also examined. As previously reported under other conditions (1,2), when root tips are transferred after 16 hours from IAA to buffer, their growth rate recovers immediately to equal or exceed that of the control. In striking contrast to this pattern, it is evident (figs 2 and 3) that the inhibition caused by ethylene is largely or completely irreversible.

Effect of Ca++. Medium A, of somewhat different composition from that of Chadwick and Burg, has been used in many previous studies on root growth in this laboratory. Omission of Ca++ from this medium causes more rapid control growth over a 16 hour period (fig 4) but growth thereafter virtually ceases unless Ca++ is present. Growth in the presence of IAA is affected by the presence or absence of Ca++ in a manner similar to the control growth (fig 4) i.e. IAA inhibition is not dependent on the presence of Ca++. It is clear however from figure 4 that when Ca++ is omitted from medium A, the degree of inhibition produced by ethylene is very greatly reduced. In fact, in the absence of Ca++, gain in weight at 16 hours is inhibited only 13% by 100 ppm ethylene, while 100 μM IAA causes 75% inhibition under the same conditions. Even in the presence of Ca++, the types of inhibition pro-

![Fig. 1. Effects of IAA and ethylene on growth of 2-day pea roots in medium B.](attachment:fig1.png)

![Fig. 2. Time course and reversibility of IAA and ethylene effects on growth of 3-day pea roots in medium B. Arrows denote times of transfer to control flasks (solid lines).](attachment:fig2.png)

![Fig. 3. Time course and reversibility of IAA and ethylene effects on growth of 3 and one-half day pea roots in medium B. Arrows denote times of transfer to control flasks (solid lines).](attachment:fig3.png)
Effects of IAA and ethylene on growth of 3-day pea roots in medium A and in medium A minus Ca²⁺.

This fact is further emphasized by figure 5, which records an ethylene response found in many of the experiments where medium A was used. Here, ethylene does not inhibit weight gain for the first 6 hours (in fact there is a slight but definite stimulation at 3 hr), but growth then ceases abruptly. By contrast, growth in the presence of IAA is essentially linear with time, in this and in all other experiments. Figure 5 also demonstrates that in medium A, as in medium B, ethylene inhibition is substantially irreversible.

Quantitative Growth Relationships. Chadwick and Burg (3) suggest that the growth inhibition caused by IAA concentrations in excess of 10 μM is of a fundamentally different nature from that occurring at lower concentrations. In support, they quote Andreae (1) as stating that root growth inhibition above 10 μM is an irreversible toxic response which can be duplicated with acetic or benzoic acids. In fact Andreae (1) showed that inhibition is fully reversible up to and including 200 μM, while a toxic response occurs only above 500 μM IAA. We find that this reversibility holds under all the conditions reported in the present paper and can therefore find no justification for drawing any such distinction at a concentration below 200 μM IAA. However, even at the supposedly ‘toxic’ concentration of 100 μM, Chadwick and Burg (3) claim that over 90% of the IAA inhibition of elongation can be explained by the tissue's response to a few ppm of ethylene (75% inhibition at 18 hr). In no case have we been able to produce an ethylene effect of this magnitude. Under the various conditions of figures 1 to 4, ethylene at 100 ppm causes 50 to 55% inhibition of elongation at 16 hours (except for the especially low inhibition, 22%, with the -Ca²⁺ treatment of fig 4). It is immediately obvious from the figures that IAA inhibition is much greater than this. Even in figures 1 to 3, where the IAA concentration does not exceed 20 μM, the ethylene inhibition is only 70 to 75% of that produced by IAA. In other experiments under similar conditions, ethylene inhibition was about 65% of that caused by 100 μM IAA. In figure 4 (medium A, + Ca²⁺) the inhibition produced by ethylene is only 50% of that given by 100 μM IAA.

It has also been reported (3) that the ratio between the absolute gain in weight and the absolute increment in length increases from a value of 1.3 in control tissue to 2.3 at 5 μM IAA and all higher concentrations, and further that this maximum value of 2.3 is also attained at all ethylene concentrations in excess of a few ppm. This similarity has been utilized (3) as further evidence to support the concept that auxin action on roots is mediated via ethylene production. Our results with IAA are presented in table I. With 5 ml of solution per

Table 1. Effect of IAA Concentration on the Weight: Length Ratio of Pea Roots

<table>
<thead>
<tr>
<th>Solution volume/ml</th>
<th>Control</th>
<th>IAA conc, μM 0.1</th>
<th>1</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.4</td>
<td>1.5</td>
<td>2.0</td>
<td>28</td>
<td>3.4</td>
</tr>
<tr>
<td>20</td>
<td>1.3</td>
<td>1.4</td>
<td>1.8</td>
<td>2.1</td>
<td>2.3</td>
</tr>
</tbody>
</table>

FIG. 4. Time course and reversibility of IAA and ethylene effects on growth of 3-day pea roots in medium A. Arrows denote times of transfer to control flasks (solid lines).
flask [as per Chadwick and Burg (3)] the weight:length ratio increased continuously, reaching a value of 3.4 at 100 μM IAA. Raising the solution volume to 20 ml gave ratios more in accord with those of Chadwick and Burg (3) but still a continuous increase with concentration was found. Furthermore, in no case did the weight:length ratio following 16 hours treatment with 100 ppm ethylene approach the IAA values at 10 or 100 μM. In figures 1 to 3 the ratios with ethylene are 1.7, 1.6, and 1.7 respectively, while in figure 4 (+Ca²⁺ treatment) the ratio is 1.4.

Ethylene Evolution. Preliminary experiments showed that in accordance with the findings of Chadwick and Burg (3) ethylene evolution from pea roots is greatly stimulated by 100 μM IAA. These authors report a maximum rate of evolution at 4 hours, followed by a progressive decline until after 16 hours the rate no longer exceeds that of the control. In our experience this pattern is most probably due to metabolism of the applied auxin. If the IAA concentration is maintained by solution renewal or if a substantially non-degradable auxin such as 2,4-D is applied, then the rate of ethylene evolution shows relatively little change over a 24 hour period.

When the effect of 100 μM IAA on ethylene evolution was examined using 2 different solution volumes, 5 ml and 20 ml, it was found (fig 6) that increasing the liquid volume per flask to 20 ml reduced the measured ethylene concentrations 2 to 3-fold. At this stage, no explanation is advanced for this somewhat unexpected finding. Calculation shows that the small additional amount of ethylene which will be dissolved in the higher liquid volume can only lead to a 2% reduction in the gas-phase concentration. The effect of solution volume is therefore a real one on ethylene production. Despite this large reduction in the rate of ethylene evolution, the change in solution volume is without effect on the inhibitory action of IAA (table II). Although both control and IAA-treated root tips grow more rapidly in 20 ml than in 5 ml, the percent inhibition produced by IAA in each case is virtually identical.

**Discussion**

It is clear from the results presented that there are serious difficulties in attempting to account for the inhibitory action of IAA on pea root growth in terms of enhanced ethylene production. From the standpoint of the kinetics and reversibility of inhibition, the quantitative growth relationships, and the influence of factors such as tissue age and Ca²⁺, there are marked discrepancies between the effects of applied IAA and of ethylene.

Ethylene evolution is undoubtedly enhanced by 100 μM IAA, and the amounts produced, about 100 nml per g fresh weight per 6 hours (fig 6), agree well with those reported by Chadwick and Burg (3) over the first 6 hours. Under our conditions (approx. 100 mg tissue, 150 ml gas phase per flask) this would yield a gas phase concentration of about 0.07 ppm ethylene after 6 hours and about 0.2 ppm after 18 hours. The data of Chadwick and Burg (3) indicate that these concentrations of applied ethylene inhibit elongation by only 20 to 30% (at 18 hr), while both their results and ours show that 100 μM IAA inhibits elongation by 75 to 80% under the same conditions. Clearly however, it is possible to suggest that the accumulated concentration of ethylene is unimportant and that inhibition is determined solely by the rate of evolution and the concentration at the site of action. In this case it would be expected that the 2 to 3-fold reduction in the rate of ethylene evolution brought about by changing the solution volume from 5 ml to 20 ml would be accompanied by some modification of the inhibitory action of IAA. Indeed, from the data of Chadwick and Burg (3) on rates of ethylene evolu-

![Fig. 6](https://www.plantphysiol.org/content/cite/1995/18/1020/gv/fig6.png)

**Table II. Effect of Solution Volume on Inhibition of Pea Root Growth by 100 μM IAA**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Control 5 ml Elongation</th>
<th>IAA 5 ml Elongation</th>
<th>Inhibition by IAA</th>
<th>Control 20 ml Elongation</th>
<th>IAA 20 ml Elongation</th>
<th>Inhibition by IAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>32.6%</td>
<td>8.8%</td>
<td>73%</td>
<td>41.2%</td>
<td>10.6%</td>
<td>74%</td>
</tr>
<tr>
<td>18</td>
<td>88.5%</td>
<td>21.8%</td>
<td>75%</td>
<td>114.6%</td>
<td>29.4%</td>
<td>74%</td>
</tr>
</tbody>
</table>
tion at different IAA levels, one might predict that such a reduction should be equivalent to lowering the IAA concentration to 10 μM. In fact however, the percent inhibition of elongation caused by 100 μM IAA is the same irrespective of solution volume (table II).

Chadwick and Burg (3) have suggested that the growth inhibition at IAA concentrations of 10 μM and below is wholly attributable to ethylene evolution, while the inhibition at higher concentrations is of an essentially different, toxic and irreversible character. It has already been noted that this assertion is partly based on a misquotation of the results of Andreae (1), and that in fact root growth inhibition is fully reversible up to 200 μM IAA. The distinction drawn by Chadwick and Burg (3) was further based on the finding that the weight:length increment ratio increases to a maximum value of 2.3 at 5 μM IAA, and that this value corresponds to that attained at ethylene concentrations above a few ppm. We, on the other hand, find that this ratio increases continuously with IAA concentration up to 100 μM (table I) while the value obtained in response to 100 ppm ethylene does not exceed 1.7. For these reasons and others previously discussed, we consider that below 100 to 200 μM there is no fundamental change in the nature of IAA inhibition and that ethylene evolution is incapable of accounting either qualitatively or quantitatively for the inhibition of root growth caused by IAA.

Literature Cited

