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Ethylene Modification of an Auxin Pulse in Cotton Stem Sections[1]

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Abstract. The effect of ethylene on the basipetal movement of indole-3-acetic acid-1-^{14}C through cotton stem sections (*Gossypium hirsutum*, L. var. Stoneville 213) was studied apart from processes involved in the uptake and exit of auxin by the section. Stem sections 60 mm in length were pretreated with ethylene or placed in room air (control) and pulse labeled for 20 min with IAA-1-^{14}C. In both the ethylene treated and control sections, the IAA-1-^{14}C taken up moved basipetally as a peak of radioactivity. Generally, the applied pulse moved down the stem sections at an average velocity of approximately 5.8 mm per hr. In some experiments, however, ethylene slightly reduced the velocity of auxin transport. Although the peak of radioactivity became broader and more dispersed during its migration through the section, it was still distinguishable after 7 hr of transport.

As the ethylene pretreatment periods were increased from 1.5 to 3.0 hr there was a progressively greater loss of activity from the pulse of applied IAA-1-^{14}C during its basipetal movement. On the average, 4% more activity was lost from the applied pulse at 1.5 hr, 15% more at 2.0 hr and 26% more at 3.0 hr when compared to control stem sections.

The data establish that ethylene inhibits auxin transport *in vivo*, and it is proposed that the effect is due possibly to increased rates of auxin immobilization.

Ethylene inhibits basipetal auxin transport in a number of plant tissues (2, 6, 10, 13, 14, 15), provided the tissue is first exposed to ethylene for a sufficient period (2, 6, 13). The method routinely employed to demonstrate this ethylene response involves the application of ^{14}C-labeled auxin to the apical cut surface of ethylene treated tissue sections. The possibility therefore exists that the apparent reduction in the amount of ^{14}C transported basipetally is due to ethylene mediated processes which occur solely in damaged cells at the cut surface. If this is the case, the effect of ethylene on auxin transport would be an artifact of the experimental system and would not occur inside of a fumigated, intact plant.

Stem sections excised from plants treated with ethylene often accumulate ^{14}C at or near the apical cut surface to a much greater extent than untreated sections (2, 13, 14). This accumulation of ^{14}C suggests that a rapid binding, conjugation or breakdown of auxin (see Review, 11) may take place at the cut surface resulting in an apparent reduction of auxin transport. Michener (12) reported that auxin destruction occurred at the cut surface of pea stem sections and ethylene treatment increased the amount of auxin destroyed. Although Michener's results could have been due to a greater amount of auxin being transported out of the control sections (12, table V), we have obtained data indicating that ethylene does enhance auxin destruction at the apical cut surface of cotton stem sections to a much greater extent than within the transport tissue (2). Further, some data indicate ethylene enhances auxin destruction by stem sections of other species (13).

In view of these results and the lack of pertinent data, the present work was undertaken to evaluate the significance of such a cut surface effect. The data presented here provide the first direct evidence that the effect of exogenously applied ethylene on auxin transport is not a cut surface phenomenon but that it does occur *in vivo*. These experiments relate to modification of auxin transport inside of the plant by exogenous ethylene and should not be confused with the question of whether amounts of ethylene adequate to cause transport inhibition are produced in unfumigated plants.

Materials and Methods

The method used to study auxin transport was essentially that of Goldsmith (7). Stem sections 60 mm in length were excised as previously described (2) from greenhouse grown 26-day old cotton plants (2) having similar internode lengths (±5 mm). Sections were selected for uniformity in stem diameter and each section was supported vertically in a plexiglass holder with the morphological apex upward. Both the apical and basal cut surfaces were

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in contact with 2 % (w/v) agar cylinders (2). For each experiment 2 such sections were placed in a chamber lined with moist toweling and were pretreated with 1000 μl of ethylene per liter of air at 30° in the dark for 1.5 to 3 hr. Two similar sections simultaneously enclosed without ethylene served as the controls.

Immediately following ethylene pretreatment, the agar cylinders on the morphological apex of each section were removed and were replaced with donor agar cylinders (2) containing 10 μM IAA-1-^{14}C (Nuclear-Chicago, 33 mc/mmole). After a 20-min uptake period, the donor cylinder on each section was removed and replaced with a replacement agar cylinder (RC_1) containing 1 μM of unlabeled IAA. After 10 min this replacement agar cylinder (RC_1) was removed and replaced with a second replacement agar cylinder (RC_2). Sections were then returned to the treatment chambers and the ethylene pretreated sections were again exposed to 1000 μl ethylene per liter of air.

After either a 2- or 2.5-hr transport period, 1 control and 1 ethylene treated section were removed from each chamber and simultaneously cut into 20 successive 3 mm segments using a specially designed multibladed cutter (precision of $\pm$0.2 mm). The remaining control and ethylene treated sections were removed from the chamber and segmented in an identical fashion after a 6- to 7-hr transport period.

Each segment, replacement agar cylinder and receiver agar cylinder was placed in a separate scintillation vial and 19 ml of Bray's scintillator fluid (3) was added. Samples were placed on a shaker for 12 hr and each vial was counted in a liquid scintillation system (Packard Tri-Carb spectrometer). The efficiency of the scintillation counting system was approximately 52 % for ^{14}C in Bray's solution. External standard channel ratio and internal standard data indicated that quenching by the control and ethylene samples was similar. Quenching by the tissue samples was slightly higher than by the agar samples.

The ^{14}C recovered from each 3 mm stem segment and receiver agar cylinder was expressed as the percent of the total ^{14}C recovered from the section plus its receiver following transport. The ^{14}C recovered from the replacement cylinders (RC_1 and RC_2) was not included in this total since the amounts of ^{14}C recovered from these cylinders of the control and ethylene treated sections were very similar. Including these replacement activities served only to reduce the percentage of ^{14}C in each stem segment and receiver. The elimination of replacement cylinder activities in no way altered the results presented here. Auxin velocities were calculated by determining the time required for the auxin pulses to move from the apical position in each section at the end of 2 or 2.5 hr of transport to the basal position after 6 to 7 hr of transport.

Over 20 pulse labeling experiments were conducted. For each pretreatment period data for 2 experiments are presented here (Fig. 1–6). These experiments had the greatest variation in results obtained between different experiments.

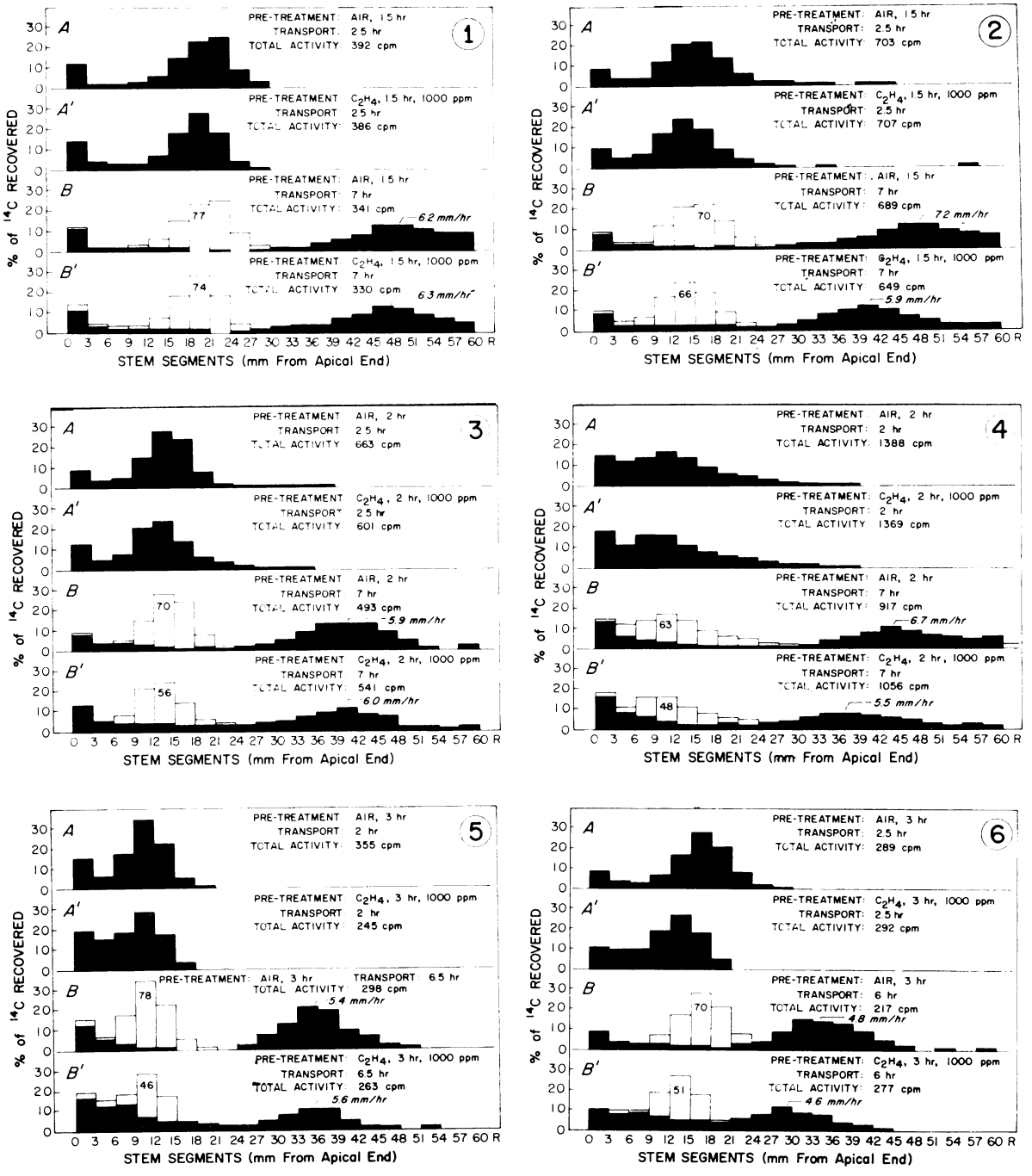
Results

Transport of a Pulse of ^{14}C-IAA for 2 to 2.5 Hours. Within 2 to 2.5 hr after stem sections were pulse labeled with ^{14}C-IAA, a peak of radioactivity has moved several mm down the sections from the point of application (Figs. 1–6, A and A'). Although the bulk (60–70 %) of the activity present in the sections was associated with this pulse, a significant percentage was retained in the more apical segments as the pulse moved down the sections. The 0 to 3 mm absorbing segment retained the highest percentage of activity.

The profiles of radioactivity in the control and ethylene treated sections were similar regardless of the length of the pretreatment period. The only significant differences were a slight lag in the movement of the peak of radioactivity in the ethylene treated sections and a slightly higher percentage of ^{14}C in the stem segments apical to this peak. No apparent relationship existed between the magnitude of the lag in transport and the length of the pretreatment period. The percentage of ^{14}C recovered in segments apical to the peak of radioactivity did increase however with increasing pretreatment periods.

Transport of a Pulse of ^{14}C-IAA for 6 to 7 Hr. After 6 to 7 hr of transport, a peak of radioactivity had moved to the basal portion of the stem sections (Figs. 1–6, B and B'). As previously noted by Goldsmith (8), all auxin molecules apparently do not move at the same rate since as the pulse of auxin moved down the sections its amplitude declined. This dispersion appeared to be non-polar and unaffected by ethylene since the peak remained relatively symmetrical in all sections. The fact that the ^{14}C-IAA taken up into the sections moved as a pulse of auxin in the control and ethylene treated sections at an average velocity of 6.0 and 5.7 mm per hr, respectively, suggested that the ^{14}C-IAA in the pulse was being actively transported.

Effect of Ethylene on the Percent of ^{14}C Transported. After a 1.5 hr pretreatment period (Figs. 1 and 2, B and B'), 77 and 70 % of the total ^{14}C in the tissue at the end of 2.5 hr of transport had moved toward the basal portion of the control stem sections, while 74 and 66 % had been transported in the ethylene pretreated sections. As the ethylene pretreatment period was increased, a correspondingly greater reduction in auxin transport occurred. Sections pretreated with ethylene for 2 hr transported 14 % (Fig. 3) and 15 % (Fig. 4) less ^{14}C than the controls while sections pretreated with ethylene for 3 hr transported 32 % (Fig. 5) and 19 % (Fig. 6)



FIGS. 1 to 6. The effect of various ethylene pretreatment periods on the basipetal movement of a pulse of ^{14}C-IAA in cotton stem sections. Stem sections 60 mm in length were pretreated with ethylene or room air for 1.5, 2, or 3 hr, pulse labeled with ^{14}C-IAA for 20 min and then cut into 20 successive 3 mm segments following various transport periods. Histograms A and A′ give the percent of the total activity recovered in each 3 mm segment and receiver agar cylinder (R) following 2 to 2.5 hr of transport. Histograms B and B′ give the same percent distribution data after 6 to 7 hr transport. Total activity includes only that activity recovered in each stem section and its receiver following transport. In each figure, histograms A and A′ have been superimposed on histograms B and B′. The number in each superimposed pulse (B and B′) represents the percent of the total ^{14}C initially present in the tissue that moved as a pulse toward the basal end of the stem section. The velocity values given in histograms B and B′ were calculated on the basis of the time required for pulses shown in histograms A and A′ to move to the position shown in histograms B and B′. The data presented in these figures represent the greatest differences between experiments which were obtained in over 20 pulse labeling experiments.

less ^{14}C. These results demonstrate that progressively less ^{14}C was transported as the ethylene pretreatment period was increased from 1.5 to 3 hr. On the average, 4 % less ^{14}C was transported at the end of 1.5 hr (Figs. 1 and 2), 15 % less at 2 hr (Figs. 3 and 4) and 26 % less at 3 hr (Figs. 5 and 6). Since the amounts of ^{14}C recovered in the control and ethylene treated sections following a given transport period were similar, the reduction in transport could not be explained on the basis of a loss of $^{14}CO_2$ by decarboxylation. The general decline in tissue radioactivity with increasing transport periods did indicate, however, that some decarboxylation occurred in both the control and ethylene treated sections (Figs. 1–6, A and A′ *versus* B and B′).

Effect of Ethylene on the Velocity of a Pulse of ^{14}C-IAA. Although ethylene treatment consistently reduced the auxin transport capacity, it did not consistently reduce the velocity of auxin transport. A 1.5 hr pretreatment period resulted in a reduction in the velocity of an auxin pulse by 1.3 mm per hr in 1 experiment (Fig. 2) whereas in a duplicate experiment (Fig. 1) there were no apparent differences. Similarly, one 2 hr pretreatment experiment revealed no apparent differences in the velocity of a pulse of auxin (Fig. 3) while another experiment resulted in a reduction of 1.2 mm per hr (Fig. 4). In contrast to these results, the 3 hr pretreatment experiments conducted in this study resulted in no significant differences in transport velocities (Figs. 5 and 6).

Apparently, the variability in the effect of ethylene on the velocity of auxin transport was related to the variability in transport velocity between different experiments. Stem sections excised from plants grown under similar but not identical conditions often transported auxin at different velocities. In Fig. 1 to 6 transport velocities differed by as much as 2.4 mm per hr for the control sections. In general it appeared that ethylene significantly reduced the velocity of auxin movement only when the rate of auxin movement in the control section was considerably above average (Figs. 2 and 4). In contrast to this variability in transport velocity obtained with greenhouse grown tissue, we have consistently obtained a small reduction in auxin transport velocity by ethylene in plants grown under controlled environmental conditions. Under these conditions the variability in transport velocity between experiments was also greatly reduced.

Discussion

The results presented in Fig. 1 to 6 provide the first direct evidence that ethylene does inhibit auxin transport *in vivo*. This is true since ethylene was shown to have an effect on auxin molecules in transit completely separated from the cut surface. Although earlier studies have shown an effect of ethylene on extractable unlabeled auxin (10, 12) and on the amount of ^{14}C transported from labeled auxin (2, 6, 13, 14, 15), they have not shown the contribution which the cut surface makes to these ethylene effects.

In Fig. 1 to 6 increased ethylene pretreatment periods resulted in a progressively greater loss of auxin from an auxin pulse in transit. Approximately 4 % less ^{14}C was transported in sections pretreated with ethylene for 1.5 hr, 15 % less at 2 hr and 26 % less at 3 hr. While a 26 % reduction in auxin transport represents only a slight modification of the auxin transport system, we (2) as well as others (6) have shown that the inhibition increases with fumigation time. Therefore, the incipient disruption of auxin transport following 1.5 hr (Fig. 1 and 2) and the increase to 26 % at 3 hr (Figs. 5 and 6) reflects the initial events which ultimately lead to a much greater disruption of auxin transport with longer fumigation periods. The experimental system used in the present study makes it difficult, if not impossible, to demonstrate a greater effect since: A) longer pretreatment periods prevent the movement of the auxin pulse, and B) longer ethylene fumigation periods following pulse labeling are limited by the length of the stem section available.

The data presented here and elsewhere (6, 14) clearly indicate that ethylene does not affect auxin transport in excised and intact tissues differently. When stem sections are excised first and the ethylene then applied, transport is disrupted in the same manner as if the stem sections were excised from ethylene pretreated plants. The important point is the length of time the tissue, whether excised or intact, is exposed to ethylene before transport is measured.

It may be argued that the pulse labeling technique used in the present report does not eliminate the influence of the cut surface from what happens to the auxin *in vivo* since all of the auxin had to pass through the apical cut surface. However, all of the inhibition which we discussed occurred after auxin pulses of similar size and shape had arrived at a position several mm past the apical cut surface of control and ethylene pretreated sections. Further, the fact that the radioactivity moved down the stem sections as a pulse for over 30 mm at an average velocity of approximately 5.8 mm per hr indicates that the migration of this pulse represents auxin transport in cotton stem sections just as it does in coleoptile sections (7). Therefore, an ethylene stimulated loss of ^{14}C-IAA from this pulse, after it is several mm past the apical cut surface, can correctly be referred to as an *in vivo* disruption of auxin transport. Furthermore, a cut surface effect of sufficient magnitude to cause a 26 % reduction in transport (Figs. 5 and 6) should result in a large accumulation of ^{14}C in the absorbing segment (0–3 mm). Yet, in Fig. 5 and 6 the absorbing segment of the ethylene treated sections contained only 3 % more ^{14}C than the controls following 2 to 2.5 hr

(Figs. 5 and 6, A and A') and 6 to 6.5 hr of transport (Figs. 5 and 6, B and B').

Burg and Burg (6), using the van der Weij "intercept technique" (16) for studying auxin transport velocity, reported that ethylene had no effect on the velocity of auxin transport in etiolated pea stem sections. In 2 of the 6 experiments of the present report, ethylene was found to cause a small but discernible reduction in the velocity of an auxin pulse removed from the influence of the cut surface. These occasional small differences in velocity may reflect true species differences, differences in experimental technique or tissue variability. It is apparent, however, that this reduction in velocity cannot account for the reduction in auxin transport since auxin transport is inhibited even when transport velocities are the same (Figs. 1, 3, 5, 6).

The data presented here (Figs. 1–6) and elsewhere (2, 6, 13) clearly demonstrate that the capacity of certain plant tissues to transport auxin progressively declines the longer the tissue is exposed to ethylene. The mechanism by which ethylene mediates this reduction however remains an enigma. The data presented in Fig. 1–6 suggest to us that increased ethylene treatments may result in a progressively greater rate of auxin immobilization (9), which may involve conjugation, binding or leakage of auxin into areas where it is no longer available for transport. A progressively greater amount of ^{14}C was retained by the transport tissue as the ethylene pretreatment period was increased from 1.5 to 3 hr. After the ^{14}C-IAA pulse had passed through the 6 to 18 mm portion of the sections, an average of 8, 10, and 8 % of the activity remained in this region for the controls while 10, 15, and 27 % remained in the same regions of the ethylene treated sections. Whatever processes are involved in this immobilization of auxin, they must occur in such a manner so as to affect only a portion of the auxin in transit because the auxin remaining in the pulse generally moves at a normal velocity and retains the same general appearance as the control pulse.

In view of the possibility that ethylene may affect membrane permeability (1), ethylene may cause auxin immobilization by stimulating the "leakage" of auxin from the transport stream into sites in the cell where it is unavailable for transport. A modification of permeability may also disrupt the structural integrity of the membranes which may in turn interfere with the normal transport of auxin molecules from cell to cell. The existence and importance of such immobilization processes however remains to be critically evaluated.

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