

# Endogenous Rhythmicity of Ethylene Production in Growing Intact Cereal Seedlings<sup>1</sup>

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## ABSTRACT

Ethylene evolution from etiolated barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), and rye (*Secale cereale*) seedlings during coleoptile growth followed a rhythmic pattern, with a period of about 16 h for barley and wheat and 12 h for rye seedlings. Leaf emergence disturbed the established rhythm of ethylene evolution.

Rhythmic phenomena play an important role in plant physiology (1, 3). The most common physiological cycles are circadian rhythms that display a periodicity of about 24 h under apparently constant environmental conditions. In some cases, a single external impulse can induce a physiological rhythm. The hypothesis proposed by Bünning requires that an endogenous self-sustaining oscillation underlies most biological rhythms (1). However, little is known about the existence of these self-sustaining oscillations in plants. In this regard, seedlings grown in continuous darkness may provide a suitable system for studying self-sustaining oscillations.

Ethylene is one of the hormones for which a circadian rhythmicity of production has been demonstrated (5, 13, 14, 16). However, only light-dark alteration-induced intact plants or detached tissues were used for these experiments.

The aim of the present study was to determine whether intact noninduced cereal seedlings produced ethylene in a cyclic fashion. We observed oscillations in ethylene production during the growth of intact etiolated barley, wheat, and rye seedlings.

## MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare* L. cv Abava), winter wheat (*Triticum aestivum* L. cv Mironovskaya 808), and winter rye (*Secale cereale* L. cv Kustro) were surface sterilized in 0.1 M KMnO<sub>4</sub> for 10 min, allowed to imbibe distilled water for 6 h, and then planted in Pyrex test tubes (12 × 120 mm, 7 mL) on moistened filter paper, one seed per tube. The seeds were germinated in the dark for 36 h. Temperature throughout these experiments was 25°C. All operations with plant material were done with a green safelight. After germination, seedlings with similar coleoptile lengths were selected for subsequent experiments. Eight seedlings were used in each group.

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Ethylene production was measured by sealing tubes with rubber caps for 2 h, allowing the accumulation of ethylene produced by individual seedlings. Ethylene in 1-mL samples was determined by a gas chromatograph (8) fitted with an alumina column and flame ionization detector. The tubes were ventilated for 0.5 h after each 2-h accumulation period.

The length of the seedlings was measured after each ethylene analysis. Two kinds of controls were used in growth studies. Cereal seedlings grown in Pyrex test tubes without periodic sealing were used as one control. As a second control, seedlings were grown in open Petri plates on a moistened filter paper.

After a 20-h dark growth period, some of the seedlings were transferred to a growth chamber with white fluorescent light (irradiance of 25 W m<sup>2</sup> for 16 h) provided by LD-40 and LB-40 lamps (Karno, USSR).

All experiments were repeated at least three times. Similar results were obtained in each experiment. The data reported in figures are from a single experiment.

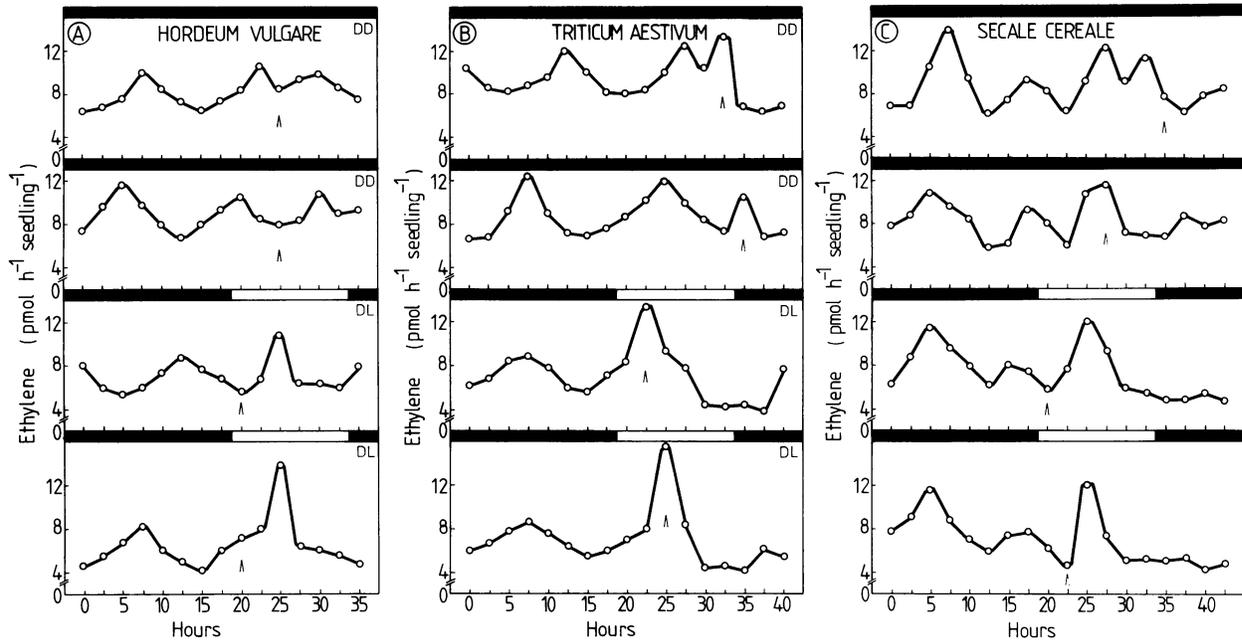
## RESULTS

### Growth of Seedlings under Different Experimental Conditions

As indicated in "Materials and Methods," test tubes were sealed with caps for 2 h, followed by 30 min of ventilation. The effect of this periodic sealing plus venting on growth was measured by comparing the growth of seedlings grown in open Petri plates. The growth of cereal seedlings grown in Petri plates was identical to that of plants grown in 7-mL test tubes, sealed or unsealed, during 62 h of the experiment (data not shown). The average time of leaf emergence from the coleoptile was identical for all of the experimental conditions used.

### Time Course of Ethylene Evolution

Figure 1, A, B, and C, shows individual rates of ethylene production by barley, wheat, and rye seedlings in continuous darkness and in 20 h of darkness followed by light. The seedlings exhibited fluctuating rates of ethylene production in the dark. A cycle of 16 h for barley and wheat and 12 h for rye seedlings was observed. Individual seedlings were not synchronous in terms of their maximum periods of ethylene evolution. In addition, the amplitude of ethylene oscillations varied significantly between individual seedlings. Switching on the light did not affect the cyclic pattern of ethylene



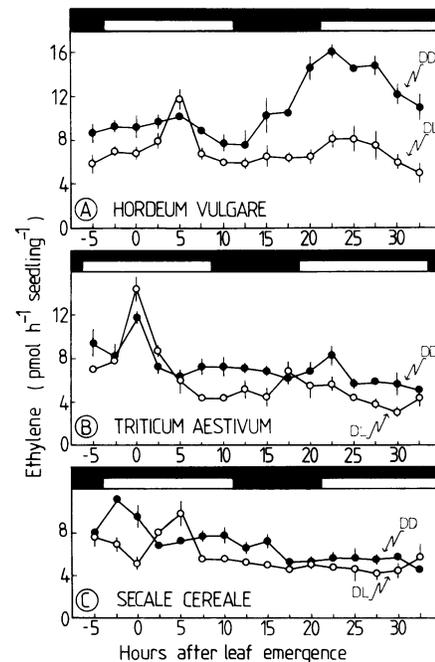
**Figure 1.** Ethylene production by barley (A), wheat (B), and rye (C) seedlings grown in test tubes in continuous darkness (DD) or transferred to light after 20 h in darkness (DL). The light conditions are indicated by an open bar (light period) and closed bar (dark period). Test tubes were closed with caps for every 2 h for accumulation of ethylene produced, followed by a 0.5-h period of ventilation. Arrows indicate the time points of leaf emergence from the coleoptile.

production. However, leaf emergence occurred 2.5 to 5.0 h after light treatment. Leaf emergence disturbed the established rhythm of ethylene evolution both in darkness and light. Because of the asynchronicity in timing, the ethylene production rate after leaf emergence from the coleoptile is shown as the mean from individual seedlings synchronized according to the time point of leaf emergence. As shown in Figure 2, leaf emergence was associated with a peak of ethylene evolution for all species examined. The data show no statistically significant differences in ethylene evolution kinetics between light- and dark-grown wheat and rye seedlings after leaf emergence (Fig. 2, B and C). In contrast, ethylene production by etiolated barley seedlings 22 h after leaf emergence had increased approximately 2-fold and had lowered again at 35 h, in comparison with light-grown seedlings (Fig. 2A).

## DISCUSSION

The results presented reveal a discernible pattern of rhythmic ethylene evolution from etiolated cereal seedlings. The oscillations appear to be endogenous and self sustained and are induced following germination rather than by external factors. In contrast, previously reported rhythmicity in ethylene production has diurnal characteristics, i.e. has been induced by light-dark alteration (5, 13, 14, 16).

It is difficult to suggest a precise localization of rhythmic ethylene production from intact cereal seedlings. The measured level may be the sum of ethylene produced by coleoptile as well as by roots and caryopses. However, it was reported that detachment-induced ethylene in rice seedlings is pro-



**Figure 2.** The mean ethylene production rate after leaf emergence from the coleoptile of barley (A), wheat (B), and rye (C) seedlings grown in test tubes in continuous darkness (●; DD) or transferred to light/dark conditions after 20 h in darkness (○; DL). Other conditions are the same as in Figure 1. Data are means from at least five individual seedlings  $\pm$  se.

duced mainly by the tip of the coleoptile (15). The disturbance of the oscillatory pattern of ethylene evolution after growth cessation in coleoptile (Fig. 2) indirectly indicated that rhythmic behavior is indeed characteristic of ethylene production in growing coleoptiles.

The question still remains, what is the physiological basis of rhythmic ethylene production during the growth of intact cereal seedlings? Circadian rhythms of certain enzymic activities and protein levels are shown to be controlled by rhythmic gene expression (4, 10). It has been suggested that oscillations in ethylene evolution by light-grown cotton seedlings reflected oscillations in its biosynthetic pathway between methionine and 1-aminocyclopropane-1-carboxylic acid (14). Alternatively, because the basal ethylene production can be regulated by various metabolic effectors (17), it cannot be excluded that fluctuations of certain effectors may drive the observed rhythmicity of ethylene evolution from cereal seedlings.

Because exogenous ethylene is a well-known inhibitor of elongation, earlier attempts have been made to correlate ethylene production with the rate of elongation growth in both intact and stressed tissues (2, 6, 7, 9). It is interesting that our preliminary results show that the periods of maximum ethylene evolution from cereal seedlings tended to coincide with periods of minimum growth (our unpublished data).

It is interesting to note that our present study shows a certain asynchronicity of individual seedlings in respect to oscillations in ethylene evolution (Fig. 1). The early observations of Liptay and Davidson (11) suggest that different embryos of germinating barley are in different physiological states. Recently, it has been shown that lack of synchronicity of individual seedlings in a representative group may disturb a typical response (12).

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