

EMANATION, PARTITIONING AND ENDOGENOUS LEVELS OF ETHYLENE IN *PHASEOLUS VULGARIS* DEVELOPING COTYLEDONS

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ABSTRACT

Experiments were carried out to determine qualitative changes in the rate of emanation and endogenous levels of ethylene in the cotyledons of *Phaseolus vulgaris* at various stages of development and using partition coefficients to test whether qualitative changes in the binding of $^{14}\text{C}_2\text{H}_4$ occur. Three phases in the rates of emanation of ethylene were observed, namely, 0–14, 14–24, and 24–48 days after flowering, respectively. The highest endogenous level of ethylene recorded was $728.7 \mu\text{l kg}^{-1}$ during the first 7 days after flowering and progressively declined until maturity. A similar trend was observed for the partition coefficient values but with high values during the 14 days after flowering. Results are indicative of a “storage system” for ethylene in *P. Vulgaris* developing seeds possibly to remove excess phytohormone from the system or to provide a reserve pool for ethylene in the cell so that levels of free hormone are maintained.

Key Words: Bean seed, $^{14}\text{C}_2\text{H}_4$, partition coefficients

RÉSUMÉ

Des essais ont été menés en vue de déterminer les changements qualitatifs du niveau endogène et du taux de dégagement de l'éthylène des cotylédons de *Phaseolus vulgaris* à différents stades de développement à l'aide de coefficients de séparation, et établir les changements qualitatifs qui s'opèrent dans la combinaison au $^{14}\text{C}_2\text{H}_4$. Trois phases de dégagement ont été observées à savoir 0–14, 14–24 et 24–48 jours après floraison. Le taux d'éthylène endogène le plus élevé observé était de $728,7 \mu\text{l/kg}$ au cours des premiers 7 jours suivant la floraison. Ce taux décroît progressivement jusqu'à la maturité. La même tendance a été observée pour les coefficients de séparation mais avec des valeurs élevées 14 jours après la floraison. Les résultats indiquent la présence d'un système de stockage d'éthylène au cours de la formation des graines de *P. vulgaris* dans le but d'éliminer l'excès de phytohormone présente dans le système ou constituer une réserve d'éthylène dans la cellule en vue du maintien des niveaux d'hormone circulante.

Mots Clés: Haricot commun, $^{14}\text{C}_2\text{H}_4$, coefficients de séparation

INTRODUCTION

One of the assumptions made in the traditional methods of sampling ethylene in higher plants is

that the rate of emanation of ethylene from a plant tissue is a reflection of the rate of synthesis. Also the effective concentration of ethylene in the cell bears a direct relationship with its rate of synthesis

and hence the physiological response (Jerie, 1978). However, research results on the metabolism of ethylene in *Vicia faba* L. (Jerie and Hall, 1978) and on the compartmentalisation of ethylene in *Phaseolus vulgaris* (Jerie *et al.*, 1979) have altered the interpretation of the ethylene status of a cell such that tissues used for emanation studies must be tested in two stages. Firstly for metabolism of ethylene, and secondly if metabolism is not detectable, the tissue must be heat-killed and then tested for compartmentalization.

It is now known that some plant tissues have a metabolic system capable of oxidizing ethylene to carbondioxide, ethylene oxide and ethylene glycol (Beyer, 1980) The simple test for metabolism of ethylene in such a plant tissue is to assay for formation of ethylene oxide since it is the primary oxidation product. In plant tissues with no ethylene metabolism, the rate of ethylene emanation is used to test for ethylene metabolism. Both ethylene emanation and compartmentalisation tests were applied to developing *P. vulgaris* seeds, with no known ethylene metabolism. This was done to demonstrate whether qualitative changes in the rate of emanation and endogenous levels of ethylene in the cotyledons of *P. vulgaris* at various stages of development are observable. Likewise, by means of partition coefficients, tests were carried out on the same cotyledons at various stages of development to demonstrate occurrence of qualitative changes in the binding of ethylene. It was envisaged that data obtained should elucidate on ethylene compartmentalisation, emanation and binding in the developing *P. vulgaris* seeds.

MATERIALS AND METHODS

Plant material. Pods of the same age and size were obtained from plants grown in a glass house. For the rate of emanation, sampling was done 7, 9, 10, 14, 20, 24, 30, 37, 44, 48 and 54 days after flowering, while for the assessment of endogenous levels and partitioning coefficients, sampling was done 8, 10, 14, 24, 36, 39, 41, 46, 50, 52, and 56 days after flowering, respectively. Harvesting of plant material was usually done in the morning during the experimental period. Pods were carefully shelled and cotyledons separated from the embryo on to a filter paper. Cotyledons weighing 0.2–0.5 g (fresh

weight) were used in each test-tube. Each age group was replicated thrice.

Ethylene emanation. Rates of ethylene emanation were determined according to the method of Jerie *et al.* (1979) in which cotyledons at various stages of development weighing between 0.2–0.5 g (fresh weight) were used, and ethylene accumulation sampled at one hour intervals. Ethylene was analysed by gas chromatography. This made it possible to establish the incubation period during which the highest rate of emanation was recorded.

Partition coefficients. Cotyledons at various ages of development were prepared as outlined above and placed in 20 ml test-tubes which were then sealed with subseal caps. After balancing the pressure, 1 ml of $^{14}\text{C}_2\text{H}_4$ (200,000 cpm) was injected into the tube using a 1 ml tuberculin plastic syringe. Samples were incubated in light for 3 hr at 20°C. At the end of incubation, 1-ml samples of gas from three replicates in each case were withdrawn using a plastic syringe and its disintegration rate measured with a Beckman Model LS-200B Scintillation Spectrometer (Jerie, 1978). After obtaining samples of free gas from above tissue, the incubation tube was opened, the cotyledons removed and the disintegration rate of the gas trapped in the tissue also determined (Jerie, 1978).

RESULTS AND DISCUSSION

Rate of ethylene emanation. Under the experiment conditions, the optimum rate of emanation was reached within 2 hr of shelling after which the ethylene concentration in the space above tissue started to decline (Fig. 1). This can be explained by considering that ethylene is initially released from both the compartment system and the tissue air spaces into free space in the closed system, this being a result of removing cotyledons from the testa and pod which are the natural physiological environments. The time lag of two hours could be explained in terms of the resistance in the diffusion path for ethylene in the tissue from the site of synthesis to the free space in the closed system. The gradual decline in the rate of emanation could also be explained by the fact that the cotyledons have been removed from the pods which in turn have been removed from

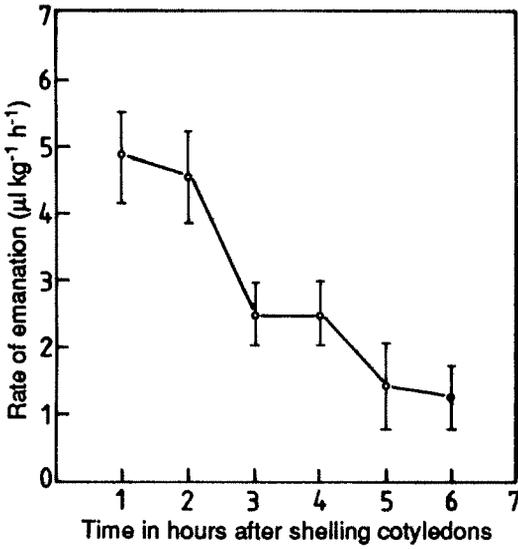


Figure 1. Time course for rate of emanation of ¹²C₂H₄ in mature cotyledons of *Phaseolus vulgaris* L.

the mother plant and, therefore, they are physiologically stressed. This means that the rate of emanation as calculated does not reflect the intrinsic rate of synthesis of ethylene in the plant cell. It is for this consideration that an extra hour

was allowed while determining rates of emanation although the emanation "peak" could be achieved within 2 hr (Jerie, 1978). Therefore, just mature cotyledons (about 25 days old) were used for determining the incubation period.

Rates of Emanation. The rate of emanation of ethylene (Fig. 2) was highest seven days after flowering (15.63 µl kg⁻¹ h⁻¹) but sharply dropped to a low level (0.7 µl kg⁻¹ h⁻¹) by the 14th day after flowering. This constitutes Phase I of the developing cotyledons. In Phase II, the rate of emanation remained rather stable up to 24 days after flowering. These two phases were followed by Phase III in which the rate of emanation gradually rose, attaining a maximum of and stabilised at about 6.6 µl kg⁻¹ h⁻¹ by the 37th day after flowering. After this period the rate appeared to decline. It is argued that these phases in the rate of emanation of ethylene may be a consequence of the developmental processes of the seed or an integral part of the growth processes of the seed.

Endogenous levels of ethylene. The endogenous levels were equally high (28.7 µl kg⁻¹) 8 days after flowering (Fig. 2), but declined to 9.30 µl kg⁻¹ by the 24th day. One salient feature of the endogenous

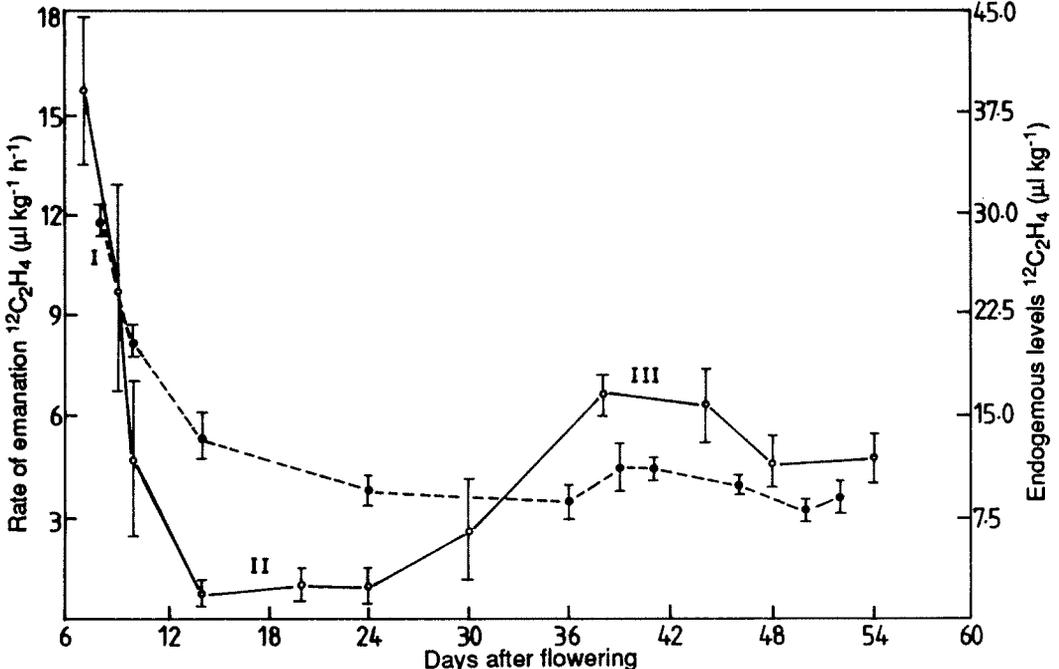


Figure 2. Changes in rate of emanation (○—○) and endogenous (●—●) levels of ethylene in developing cotyledons of *Phaseolus vulgaris* L. I-III are Phases of emanation.

levels is that at every developmental stage, the rates of release of the endogenous amounts were far higher than the observed rates of emanation.

Rate of accumulation of $^{14}\text{C}_2\text{H}_4$ and partition coefficients. A maximum rate of accumulation of $^{14}\text{C}_2\text{H}_4$ in the developing cotyledons was achieved within 1 hr and amounted to $5.5 \times 10^3 \text{ dp mg}^{-1}$ (Fig. 3). It is suggested that at the end of the two-hour period an equilibrium is attained (Jerie, 1978). A similar observation was made by Bengochea (1987). The rate of accumulation of $^{14}\text{C}_2\text{H}_4$ in the cotyledons of *P. vulgaris* at various ages of development and partition coefficients derived from the data were maximum on the 12th day after flowering after which they dropped (Fig. 4).

From the data on partition coefficients two peaks are observable, namely, between 9 and 22 days after flowering, and then between 22 days and 42 days after flowering. The Cp values and rate of accumulation of $^{14}\text{C}_2\text{H}_4$ at various stages of development (Fig. 3) agree with the values obtained by Jerie (1978) and Jerie *et al.* (1979).

In general, the data indicate that ethylene status is dependent on the developmental stage of *P. vulgaris* seed. It could be speculated that a signal is received by the receptor cells at the site

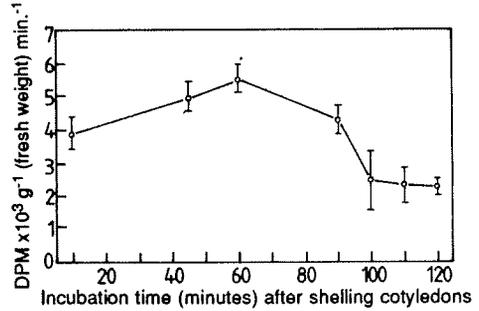


Figure 3. Time course for rate of accumulation of $^{14}\text{C}_2\text{H}_4$ in *Phaseolus vulgaris* L. The amount accumulated per unit time is plotted against "incubation time". Samples were incubated with $10,000 \text{ DPM ml}^{-1} \text{ } ^{14}\text{C}_2\text{H}_4$.

of synthesis resulting in acceleration or deceleration of the rate of synthesis depending on the developmental stage of cotyledons. With respect to the view that ethylene is a regulatory factor in growth, an interesting question arises: are the fluctuations in endogenous levels of ethylene, *per se*, in space and time with the result that the rate of synthesis is altered, the primary

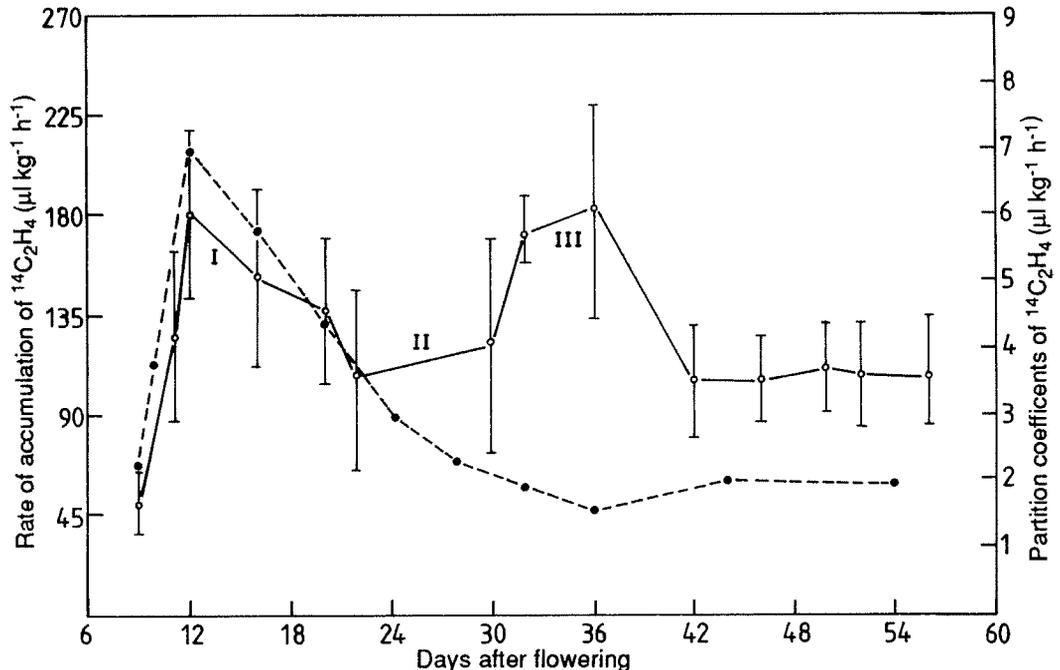


Figure 4. Changes in accumulation (—●—) and partition coefficient (---○---) of $^{14}\text{C}_2\text{H}_4$ in developing cotyledons of *Phaseolus vulgaris* L. Bars indicate standard errors.

control of growth, or is it the effect of the hormone exerted at the active site of action of the ethylene molecule? These questions merit further investigations as the data obtained do not answer them. Nevertheless, the endogenous levels of ethylene were quite high. Two possible functions of the compartmentalising system is suggested. One of these could be to remove excess gaseous hormone from high and rapid turnover of the ethylene precursor 1-Amino acid-1-carboxylic acid (ACC) in the cell. The second function could well be to provide a reserve pool for ethylene in the cell so that levels of free hormones are maintained. The underlying assumption in this case is that changes in the critical levels of the hormone is the mechanism of action of ethylene. Investigations by Bengochea (1981) also showed that the testa, pod, abscission zones, roots and stems in *P. vulgaris* have a capacity to compartmentalise ethylene. This then points to the physiological significance of the compartmentalising system in leguminous plants (Jerie *et al.*, 1979).

In conclusion, it is proposed that the physiological function(s) of the storage system of ethylene in *P. vulgaris* could further be evaluated by measurement of ethylene emanation in cotyledons of various stages of development to establish whether ethylene emanations together with the endogenous levels are in agreement with the turn-over rate of ACCS. Since ethylene is involved in many plant growth processes, biotechnological studies aimed at identifying the genome (Deragon and Landry, 1992; Majiwa *et al.*, 1993; Joshi and Nguyen, 1993) coding for ethylene production, action and metabolism will elucidate on the storage system of ethylene.

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