Plant Physiol. (1976) 57, 480–485

Studies on the Carbon Dioxide Promotion and Ethylene Inhibition of Tuberization in Potato Explants Cultured *in Vitro*[1]

Received for publication July 28, 1975 and in revised form October 27, 1975

ANGEL M. MINGO-CASTEL, ORRIN E. SMITH,[2] AND JUNJI KUMAMOTO
Department of Plant Sciences, University of California, Riverside, California 92502

ABSTRACT

Ethylene inhibited the tuberization of etiolated potato (*Solanum tuberosum* L. var. Red La Soda) sprout sections cultured *in vitro*. Carbon dioxide did not overcome the C_2H_4 inhibition but it was required for normal tuberization. Ethylene totally prevented root formation and development. It inhibited stolon elongation, and caused thickening and diageotropical growth of the stolon. In addition, C_2H_4 prevented the accumulation of both starch and red anthocyanin which are always present in a tuber. Ethylene also inhibited the kinetin-increased tuberization of sprout sections.

Three to five days of exposure to CO_2 were required to obtain promotion of tuberization of stolons cultured *in vitro*. Bicarbonate ion did not affect starch synthetase activity isolated from potato tubers *in vitro*. The evidence presented suggests that CO_2 gas rather than HCO^-_3 or CO^{2-}_3 ions in equilibrium with dissolved CO_2 was probably responsible for the stimulation. Morphological changes elicited by CO_2 and C_2H_4 are described and the mechanism of action of both on tuberization is discussed.

A number of apparently contradictory reports in the literature on the role of ethylene in tuberization were discussed previously (16). Potato plants sprayed with Ethrel registered a decrease in the number of tubers/plant (12), or there was no change in either the number of tubers/plant or the weight of tubers (20). Singh (20) noticed phytotoxic effects from sprays containing Ethrel concentrations greater than 500 mg/l. Bodlaender (3) showed that Ethrel sprays on potato plants may produce quite different results, depending on the concentration of Ethrel and time of harvest. In only two cases did he find tuberization favored by Ethrel sprays: slight increases were obtained in tuber yield at early harvest time for a narrow range of Ethrel concentrations, and in the number of tubers/plant at regular harvest time. For other concentrations and time of harvest, there was either no change or a decrease in tuberization.

Ethrel has been reported to increase tuberization of etiolated potato sprouts cultured *in vitro* (8). We have recently reported on the actions of ethylene and CO_2 on tuber initiation of isolated potato stolons cultured *in vitro* (16). A promotion of tuberization by CO_2 and an inhibition of the CO_2 stimulation by C_2H_4 was demonstrated. Ethylene had, by itself, an inhibitory effect on endogenous tuberization and on kinetin-induced tuberization of stolons. The present work provides additional information about the physiology and morphology of the CO_2 induction and the C_2H_4 inhibition of tuberization *in vitro*. The earlier work on potato stolons has been further extended to the study of the actions of CO_2 and C_2H_4 on tuberization of sprout sections cultured *in vitro*.

MATERIALS AND METHODS

Plant Material and *in Vitro* Culture. Tubers of *Solanum tuberosum* L. var. Red La Soda which had been grown for seed were used in all experiments. On one occasion, var. White Rose was also tested. Sprout sections or stolons were obtained according to the technique described previously (16) with a few modifications. Excised stolons grown from axillary buds of sprout sections were surface-sterilized by immersion in a Purex bleach solution (diluted 1:10), for 2 to 3 min, before being transferred to culture flasks or test tubes. Where indicated, kinetin was used at a concentration of 5 mg/l. Tuberization of sprout sections was studied by culture in medium 2 using the methodology described by Mingo-Castel *et al.* (16). When used, CO_2 and C_2H_4 were injected in amounts which would produce initial concentrations in the flasks of 10% (v/v) of CO_2 and 5 μl/l of C_2H_4. Filter paper was inserted in small (16 × 41 mm) test tubes before autoclaving the flasks containing the culture medium. Trapping solutions (16) for CO_2 and/or C_2H_4 were dispensed later in the small test tubes.

Time Course Experiments of CO_2 Action. The following changes were made in relation to the methodology described previously (16). Sprouts were excised when they were 6 to 8 cm long. Two ml of sterile H_2O were added to culture flasks on top of the solidified culture medium 2 to prevent desiccation of the internal atmosphere in the flasks produced by absorption of H_2O by a 20% (w/v) KOH solution (CO_2 trapping solution).

One-third of the available stolons was cultured in medium 2 with 10% (v/v) CO_2 in the absence of C_2H_4 for 30 days. Another third was cultured in the same conditions for 1, 2, 3, or 5 days and then transferred (designated as tr) to medium 2 in a flask covered with a Morton stainless steel closure which allowed exchange with external air (open control), or the flasks were purged (designated as p) under vacuum in the sterile transfer hood for 3 min and then covered with a Morton closure. After purging, the CO_2 concentration in the flasks was less than 0.2%. The small test tube containing mercuric perchlorate solution was removed from the flask at this time. The final third of the stolons was cultured in medium 2 in an atmosphere in equilibrium with the exterior (open control).

Extraction and assay of starch synthetase from potato tubers were based on the procedure described by Hawker *et al.* (10).

RESULTS

Effect of C_2H_4 and CO_2 on Tuberization and Rooting Development of Sprout Sections Cultured *in Vitro*. All experiments were carried out with sections having a high tuberization potential. After 30 days of incubation, 83% of the sections formed a

[1] This paper represents part of the Ph.D. dissertation of A. M. Mingo-Castel.

[2] Present address: Department of Horticulture, Washington State University, Pullman, Wash. 99163.

tuber (Fig. 1). The tuberization percentage obtained in the absence of both gases ($-CO_2$ $-C_2H_4$) was increased by 25% in the presence of CO_2 ($+CO_2$ $-C_2H_4$) and decreased by 34% in the presence of C_2H_4 ($+C_2H_4$ $-CO_2$). Ethylene inhibition was not overcome by the presence of CO_2. When compared with the endogenous tuberization of sections (open control) C_2H_4 exerted an inhibition of 66%.

Ethylene totally prevented root formation both in the presence or absence of CO_2 (Table I). Even though root primordia were easily visible in 50% of the sections, they never developed into roots if C_2H_4 was present. Stolon elongation was decreased 7.5-fold by the presence of C_2H_4 and 24-fold by the presence of both C_2H_4 and CO_2.

Ethylene elicited several morphological modifications which were readily apparent when the sprout sections cultured in the open control (Fig. 2A) were compared with the sections cultured under C_2H_4 treatment in the absence of CO_2 (Fig. 2, C and D). Ethylene strongly retarded stolon elongation but, when it did occur, growth was diageotropic and produced a stolon thicker than usual.

A red skinned potato (var. Red La Soda) was selected because it gives an additional valuable and easily recognizable feature associated with tuberization: a localized red color of the tuber at the locus of tuberization which is clearly distinguishable against the white stolon. Periodic inspection of the Red La Soda stolons during culture revealed the following sequence of events occurring at the locus of tuber initiation: (*a*) starch accumulation, (*b*) visible swelling, and (*c*) red pigment accumulation. When working with stolons, starch accumulation was first detected after 4 days of culture. The first swellings were observed after 7 days,

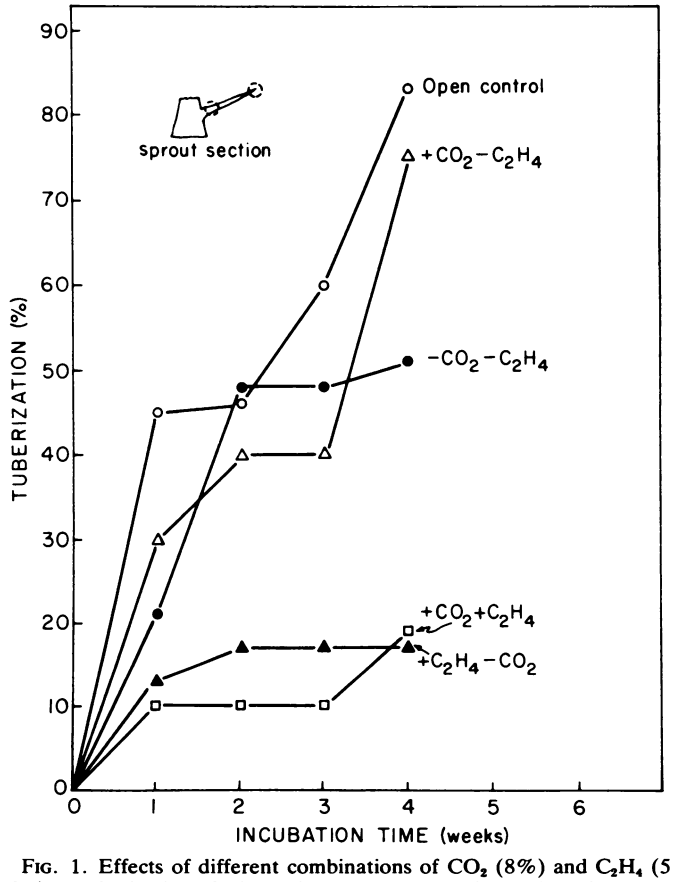

FIG. 1. Effects of different combinations of CO_2 (8%) and C_2H_4 (5 μl/l) on tuberization of etiolated potato sprout sections cultured *in vitro*. The results shown represent the weighted means from three experiments with similar results using 10 potato sections (var. Red La Soda) per treatment.

Table I. *Effect of Ethylene and Carbon Dioxide on Percentage of Root Formation and Stolon Length of Cultured Sprout Sections*

Sections were cultured at 25 C under different combinations of CO_2 (10%) and C_2H_4 (5 μl/l) for a period of 30 days. The results represent the weighted means from three experiments with similar results using 10 sections per treatment. Stolon length was measured after 30 days of incubation.

Treatments	Root formation (%)			Stolon length (mm)
	Incubation time (days)			
	8	14	30	
Open control	80	93	93	52.5
CO_2 minus C_2H_4	70	80	80	10.9
C_2H_4 minus CO_2	0	0	0	5.7
Minus CO_2 minus C_2H_4	74	83	83	42.7
CO_2 plus C_2H_4	0	0	0	1.8

and the appearance of a weak pink color after 10 days of culture. The tuber skin color intensifies progressively from a weak pink to a deep red. Figure 2, C and D, shows the results of C_2H_4 inhibition of tuberization in different sprout sections at two different stages in the process of endogenous tuberization at the time of excision. In Figure 2C there is a slight pink swelling at the base of the stolon, whereas in Figure 2D the protuberance is red and in an advanced state of development, but yet C_2H_4 has prevented tuberization in a normal sense.

The simultaneous presence of C_2H_4 and CO_2 almost totally inhibited stolon growth and resulted in extensive proliferation of callus throughout the surface of the sprout section (Fig. 2B). Tuber formation was prevented by C_2H_4. An illustration of the C_2H_4 action may be seen in Figure 3A where a pink colored swelling was terminated by a white apex. A longitudinal midsection was made through the thickened stolon and it was stained with I_2 + KI solution. The pink base contained starch but the white upper region did not. Carbon dioxide, in the absence of C_2H_4, promoted leaf development and expansion at the stolon tip, and yielded violet colored tubers rather than red tubers (Fig. 3B).

Elsewhere (16) we reported inhibition by C_2H_4 of the kinetin-induced tuberization of stolons cultured *in vitro*. Swellings promoted by kinetin were visible after 7 to 10 days in culture. Figure 4 shows a "hybrid" form of swelling as a result of competition between kinetin and C_2H_4 after 9 days in culture. At the base, a spherical type of swelling characteristic of normal tuberization was observed, but it was continued in the upper part by a thickened stolon typical of C_2H_4 treatment. A tightly closed apical hook, which is a very typical morphological characteristic of C_2H_4 action, was observed.

The tuberization percentage of etiolated potato sprout sections cultured *in vitro* in the absence of kinetin or any other growth regulators (endogenous tuberization potential) responds to the presence of 5 mg/l kinetin in the medium with a 30% increase (unpublished results). We found an inhibition of this kinetin-promoted tuberization by 5 μl/l of C_2H_4. The morphology of the C_2H_4-inhibited swellings was very similar to the inhibition by C_2H_4 in the absence of kinetin (Fig. 3A) though the stolon length was even shorter.

Time Required for CO_2 Action on Tuberization. After 14 days of culture under CO_2 in the absence of C_2H_4, about 70% of the tubers eventually produced after 30 days of incubation, were already visible as swellings (16). This suggested that the promotive action by CO_2 took place some time before 14 days of culture. An experiment was designed to determine the minimum length of time of exposure to 10% CO_2 in the absence of C_2H_4

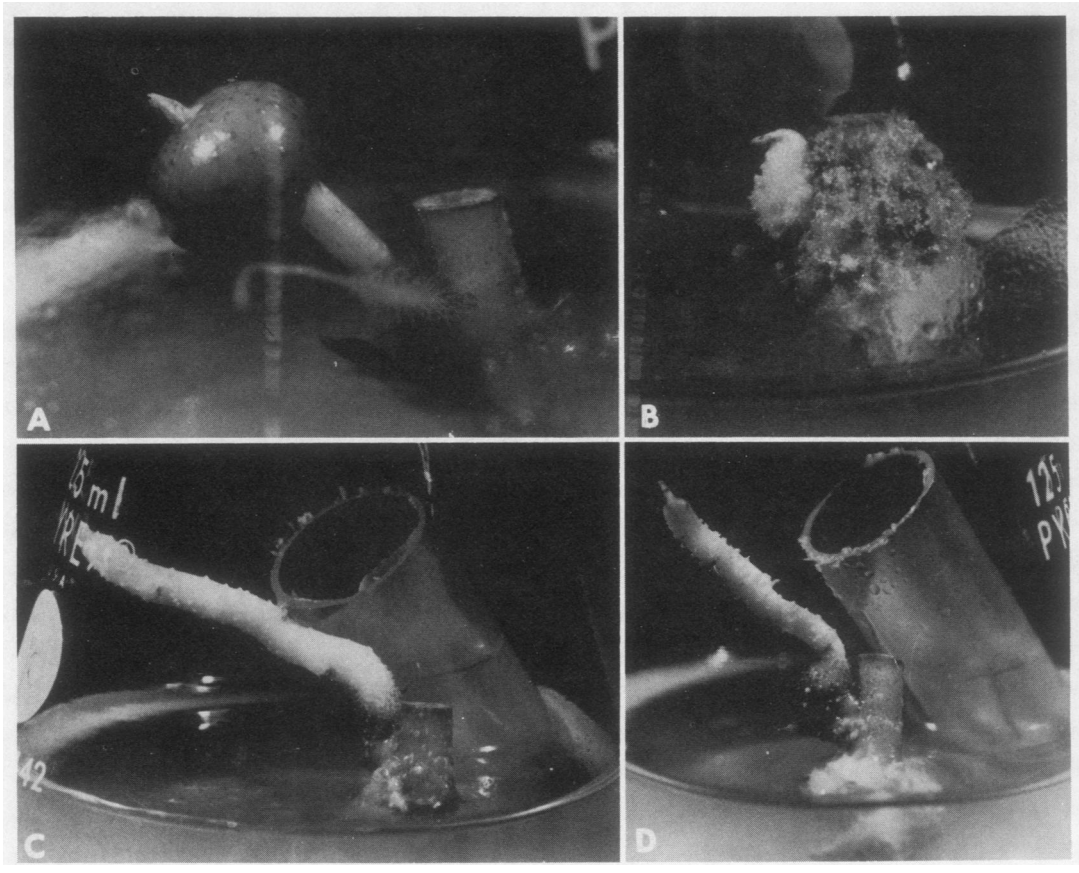

FIG. 2. Morphology of a potato sprout section (var. Red La Soda) after 30 days of culture under different conditions: A: Open control; B: 5 μl/l C_2H_4 plus CO_2; C: 5 μl/l C_2H_4 minus CO_2; this section was at an early stage of endogenous tuberization at the time of excision from the sprout; D: 5 μl/l C_2H_4 minus 10% CO_2; this section was at an advanced stage of endogenous tuberization at the time of excision from the sprout.

required to obtain the same promotion of tuberization as continuous exposure for 30 days (Figs. 5 and 6). Whether the stolons were released from CO_2 treatment by a transfer to another flask (Fig. 5) or by a purging operation (Fig. 6), 3 to 5 days were sufficient to trigger the observed CO_2 promotion of tuberization.

Study of CO_2 Tuberization Response in Stolons Cultured *in Vitro*. We reported a stimulation of tuberization in potato stolons by 8% CO_2 (16). Subsequently, it was found that a similar response was obtained from concentrations of CO_2 ranging from 1.25% to 20% in the absence of C_2H_4. These values represent initial v/v concentrations of CO_2 in the flasks immediately after injection.

In order to determine which form of CO_2, (H_2CO_3, HCO^-_3 or CO^{2-}_3) was responsible for promotion of tuberization, the stolons were incubated in culture medium as usual but the pH was adjusted to 3 or 5.7. A concentration of 2.5% (w/v) agar was necessary at pH 3 to obtain solidification of the medium after cooling to 25 C. An initial concentration of 10% CO_2 was selected for this study. The endogenous threshold tuberization of 20% obtained in the control treatments was raised to 60% under 10% CO_2 treatment in the absence of C_2H_4 at both pH 5.7 and pH 3 (Table II). Since only 5 days were sufficient to trigger the CO_2 response, we can calculate an upper limit to the concentration of CO_2 gas and the other forms of dissolved CO_2 in the culture flasks responsible for promotion of tuberization by finding its concentration after 7 days of incubation. The CO_2 concentration inside the flasks was monitored by gas chromatography at weekly intervals during a period of 3 weeks; controls containing no stolon inside were also monitored. Assuming that the solubilities of CO_2 in H_2O and in the culture medium are the same, and that after 7 days of incubation an equilibrium has been reached, the amount of CO_2 dissolved into the medium after 7 days can be calculated in two independent ways. One value can be obtained from the actual concentration and partial pressure of CO_2 measured by gas chromatography after 7 days; the other by calculating the CO_2 lost in the internal atmosphere of the flasks since the time of injection. In the second approach, the CO_2 released by respiration was taken into account by comparison with the flasks without stolons. The agreement between the two calculations was reasonable: 4.81 ml and 5.12 ml of total CO_2 dissolved, which represent 32% and 34% of the total CO_2 contained in the flask. From these values, considering the hydration reaction of CO_2 and the dissociation of H_2CO_3 into HCO^-_3 and CO^{2-}_3, the concentrations of the ionic species were calculated. The smallest CO_2 concentration in the internal atmosphere measured from injection time until the 7th day was 6.35%. One or more of the following forms at the concentrations obtained should be responsible for the promotion of tuberization: $[HCO^-_3] \leq 0.24$ μM; $[H_2CO_3] \leq 0.57$ mM; $[CO_2] \geq 6.35\%$ (v/v).

In another experiment we studied the effect of HCO^-_3 on the activity of starch synthetase isolated from potato tubers *in vitro*. No change in activity by HCO^-_3 was found.

DISCUSSION

Since the most striking result from preliminary experiments was the strong inhibitory action of C_2H_4 on tuberization, it seemed important to test the extent of the inhibitory action in sprout sections which have a high endogenous tuberization potential.

We know that the percentage of tuberization of etiolated sprout sections cultured *in vitro* depends mainly on the physiological age of the mother tuber, the length of the sprout at the time of excision, and the relative position of the bud-containing

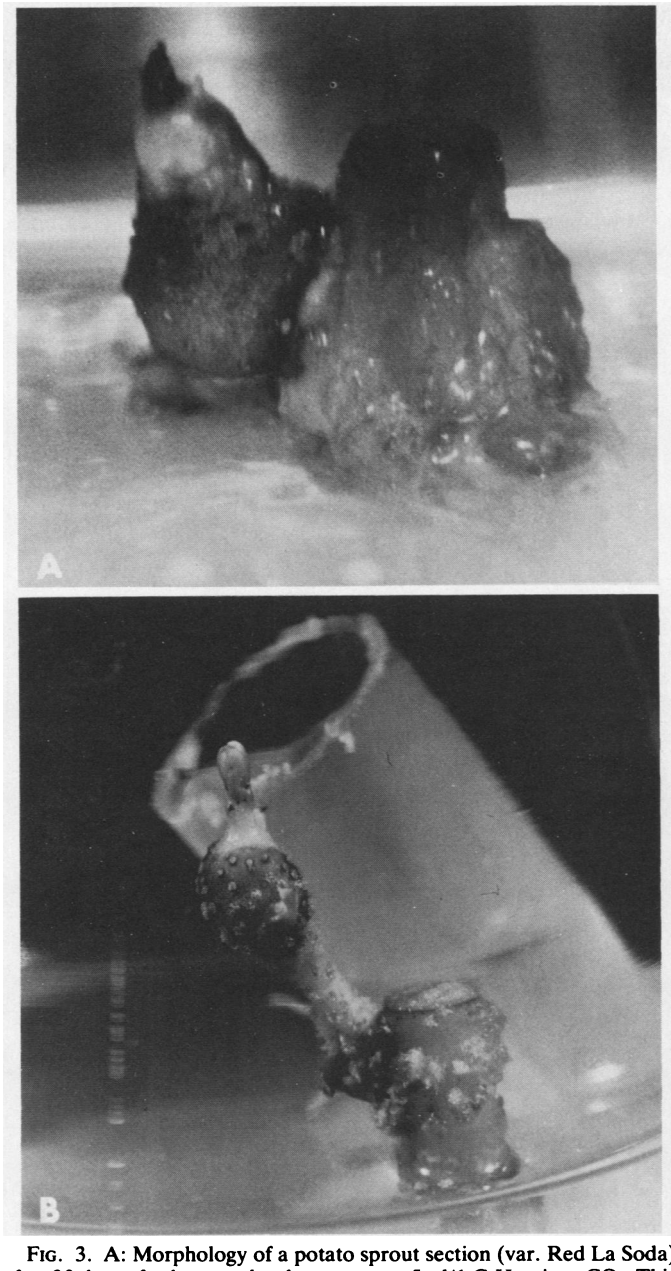

FIG. 3. A: Morphology of a potato sprout section (var. Red La Soda) after 30 days of culture under the treatment 5 μl/1 C_2H_4 minus CO_2. This section was at an *advanced* stage of endogenous tuberization at the time of excision from the sprout. Note the incompletion of the tuber originated by C_2H_4 action. B: Foliar expansion at the stolon apex of a potato sprout section (var. Red La Soda) after 30 days of culture under the treatment 10% CO_2 minus C_2H_4.

section in the sprout (unpublished results). Tuberization increases with age and it is higher in the lower portions of the sprout. This pattern of endogenous tuberization may be explained by the upward movement of a tuberizing stimulus from the mother tuber through the sprout. Several pieces of evidence support the idea of the presence in seed pieces of such stimulus (6, 14) which can linger or persist in storage at least through four generations (7). At advanced physiological stages, the lower regions of stems tuberize more readily than the upper regions (13).

Ethylene and CO_2 showed opposing effects on the tuberization of sprout sections *in vitro* (Fig. 1). Ethylene was a strong inhibitor of tuberization and CO_2 did not overcome this inhibition. Carbon dioxide slightly promoted tuberization when compared to gas-trapped controls. We obtained a similar percentage of tuberization in the 10% (v/v) CO_2 treatment in the absence of C_2H_4 as in the open control where there was free gas exchange with the external atmosphere. This indicated that a sufficient level of CO_2 was present in the normal tuberization of sprout sections. Also a comparison of those treatments with the culture under the absence of both gases indicated that a total absence of CO_2 is detrimental for tuberization and that a certain level of CO_2 is required. Unlike its effect on *in vitro* cultured stolons (16), CO_2 did not increase the percentage of tuberization over the percentage resulting from the endogenous tuberization potential nor did it partially overcome the C_2H_4 inhibition.

Generally C_2H_4 exerts its inhibitory effect by totally preventing swelling of the growing stolon, and in some instances, where the tuberization stimulus present in the sprout section has already triggered the formation of a tuber at the time of excision and culture, the inhibitory action is revealed through an interference on the development of the tuber resulting in a morphologically incomplete tuber.

Root formation, which was depressed by C_2H_4 in cultured stolons (16), did not occur at all in cultured sprout sections in the presence of C_2H_4. We reported, recently, on the partial inhibition of tuberization of stolons cultured *in vitro* by C_2H_4 (16). Potato sprout sections, also "sense" the presence of C_2H_4 since they responded by showing some of the typical morphological symptoms of C_2H_4 action: inhibition of longitudinal stolon growth, thickening of the growing stolon, diageotropism, and

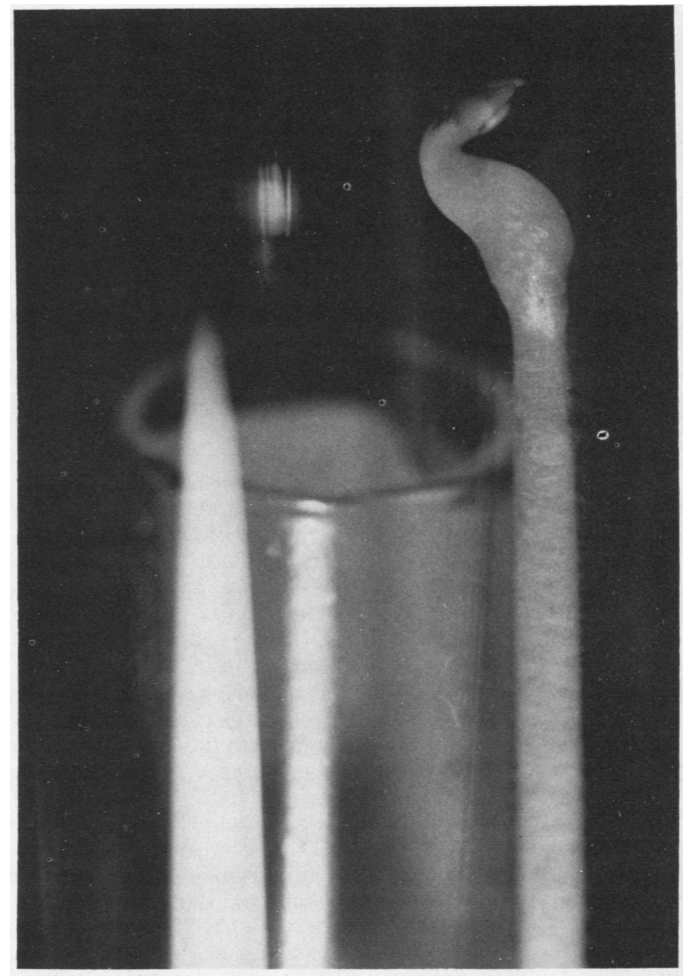

FIG. 4. Hybrid type of swelling at the subapical region of a potato stolon (var. Red La Soda) as a result of the competition between the actions of kinetin (5 mg/1) and C_2H_4 (5 μl/1) after 9 days of culture.

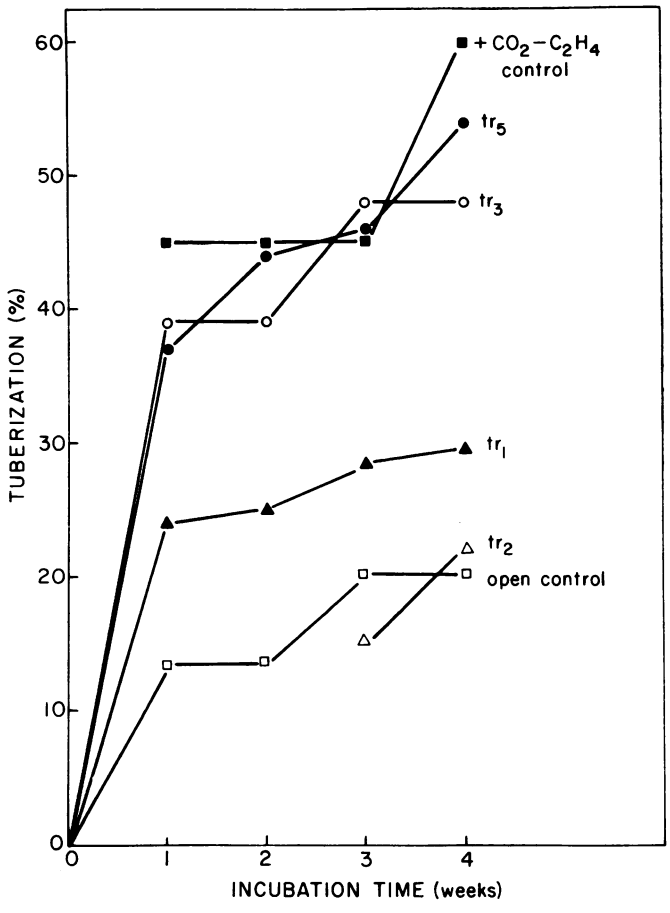

FIG. 5. Tuberization percentage of potato stolons (var. Red La Soda) cultured under the treatment 10% CO_2 minus C_2H_4 for 1, 2, 3, or 5 days, and then transferred to an open control. Part of the stolons were continuously cultured under 10% CO_2 minus C_2H_4 for 30 days. tr: transferred; p: purged.

inhibition of root formation. In addition to these characteristics, C_2H_4-treated sprout sections showed a sharp decline in tuberization percentage. Tubers were not initiated at all, or if they were, the formed swellings could not complete the necessary development to become tubers. Furthermore, C_2H_4-induced swellings did not contain starch nor did they show the red anthocyanin pigment always present in the tubers of red potato varieties. Moreover, C_2H_4 inhibited the increased tuberization of sprout sections which normally occurs in the presence of kinetin.

The mechanism of action whereby C_2H_4 exercises its hormonal control over a wide variety of physiological responses is at the present time unknown, though a number of partial effects on metabolism have been reported (1). The mechanism of C_2H_4 action cannot be explained as a simple physical effect on membranes (15); and C_2H_4 was found to have no effect on water permeability in potato tissue (18). Since gas exchange between internal and external atmosphere in potato tubers is fairly rapid (4), problems of diffusion through membranes would not appear to be important.

The time of treatment with CO_2 required to stimulate tuberization of stolons is 3 to 5 days. Since the process of transfer of stolons, by itself, had no effect on tuberization and we obtained similar tuberization responses for both "transfer" and "purging" techniques, CO_2 gas rather than dissolved HCO^-_3 or H_2CO_3 is probably responsible for the increase in tuberization. We have calculated the concentrations of the active species which would originate from dissolved CO_2 after 7 days of incubation. Since only 5 days are necessary to trigger CO_2 action, the calculated values represent upper limits for the concentrations of the species involved. The concentration of dissolved bicarbonate (0.24 μM) at pH 3 is almost negligible compared to the concentration of carbonic acid (0.57 mM), and it would be unlikely to have a relevant role in the promotion of tuberization. Carbonic acid does not appear to be responsible for this promotion either since the calculated concentration is very similar to the concentration of carbonic acid reported by Burton (5) in potato tuber. According to Burton, the normal concentration of dissolved carbonic acid in sap of potato tubers maintained in the open atmosphere

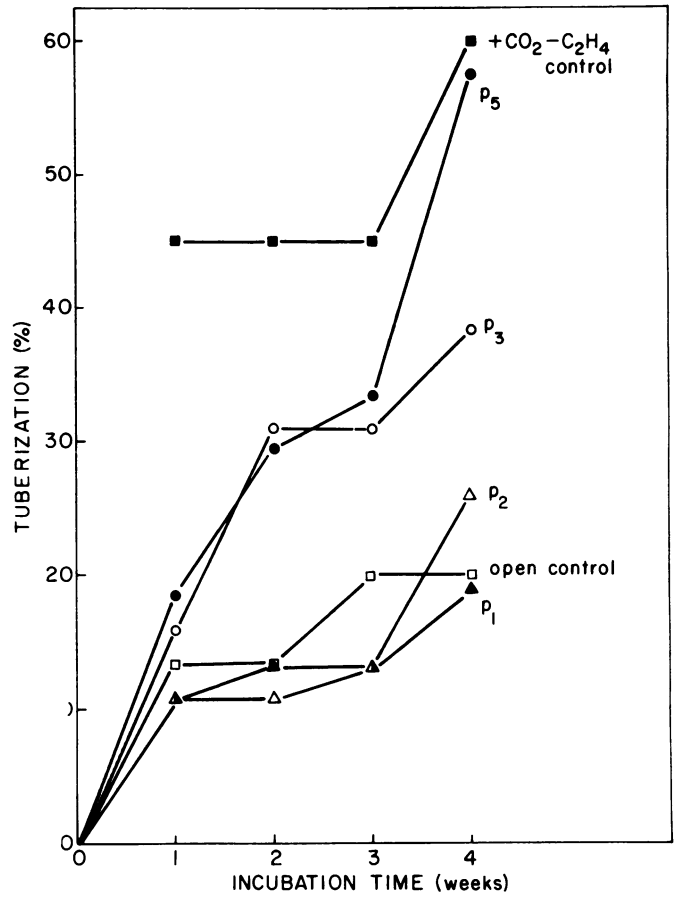

FIG. 6. Tuberization percentage of potato stolons (var. Red La Soda) cultured under the treatment 10% CO_2 minus C_2H_4 for 1, 2, 3, or 5 days; then the internal atmosphere in the flasks was purged and culture was continued as in open control. Part of the stolons were continuously cultured under 10% CO_2 minus C_2H_4 for 30 days. p: purged.

Table II. *Effect of Ethylene and Carbon Dioxide on Final Tuberization Percentage of Potato Stolons Cultured at Two Different pH*

Sections were cultured at 25 C under different combinations of CO_2 (10%) and C_2H_4 (5 μl/l) for a period of 30 days. The results represent the weighted means from two experiments with similar results using 10 stolons per treatment. When used, kinetin concentration was 5 μg/ml.

Treatment	pH	Tuberization
		%
CO_2 minus C_2H_4	3	59
Minus CO_2 and C_2H_4	3	20
Minus CO_2 and C_2H_4, plus kinetin	3	56
Open control minus C_2H_4	3	21
CO_2 minus C_2H_4	5.7	60

at 25 C is 2 mM, and the concentration of CO_2 gas in the intercellular spaces is 7% (v/v). Culture of the potato stolons in an atmosphere containing a high concentration of CO_2 will increase the partial pressure of CO_2 in the intercellular spaces. While the first detectable event in tuber initiation is starch accumulation, bicarbonate did not affect starch synthetase activity isolated from potato tubers *in vitro*. Carbon dioxide gas, then, seems to be the most likely candidate for promoting the tuberization response. Carbon dioxide would play a hormonal role on this system exerting its control at a biochemical or biophysical level in the cell. There is substantial evidence indicating that increased concentration of CO_2 may be responsible for changes in solubility, dissociation, localization of charge in the cell environment, and changes in plasticity and permeability of membranes (17). Usually there is no more than 5% (v/v) CO_2 in the soil atmosphere (22) though under exceptional conditions it may reach 7.7% (21).

Tuberization is brought about by a change in polarity of cell enlargement in the subapical region of the stolon. It is important to notice the similarities and differences between the actions of C_2H_4, CO_2, and kinetin on this region. Ethylene has been shown to cause lateral growth in the subapical region of etiolated pea stems due to a change in the pattern of deposition of microfibrils from transverse to longitudinal in response to C_2H_4 treatment (2, 19). Kinetin also promoted radial cellular expansion but there is evidence in favor of a different mechanism of action (2). Ethylene inhibited DNA synthesis in subapical tissue segments of etiolated peas, and this inhibition was reversed by benzyladenine (11). In etiolated bean hypocotyls (9) individual small growing zones responded differently to hormone treatment and each hormone acted specifically on a particular zone. Ethylene and CO_2 had opposite effects in every zone of the hypocotyl. From the above evidence we propose that the antagonism between the promotive actions of CO_2 and kinetin in tuberization and the inhibitory action of C_2H_4 may lie partially at the intercellular level as a result of an action on the pattern of deposition of microfibrils or the connection of microtubules in the subapical region of the potato stolon.

Acknowledgments – The authors wish to thank T. Hankins of Agri Empire, San Jacinto, California for providing the potatoes used in this study and J. Moore for making the illustrations.

LITERATURE CITED

1. ABLES, F. B. 1973. Ethylene in Plant Biology. Academic Press, New York.
2. APELBAUM, A. AND S. P. BURG. 1971. Altered cell microfibrillar orientation in ethylene-treated *Pisum sativum* stems. Plant Physiol. 48: 648–652.
3. BODLAENDER, K. B. A. 1972. De invloed van groeiregulerende stoffen op aardappelen. Bedrijfsontwikkeling 3: 595–601.
4. BURTON, W. G. 1950. Studies on the dormancy and sprouting of potatoes. I. The oxygen content of the potato tuber. New Phytol. 49: 121–134.
5. BURTON, W. G. 1951. Studies on the dormancy and sprouting of potatoes. II. The CO_2 content of the potato tuber. New Phytol. 50: 287–296.
6. CHAPMAN, H. W. 1958. Tuberization in the potato plant. Physiol. Plant. 11: 215–224.
7. CLAVER, F. K. 1971. Formación de cuatro generaciones de tubérculos sin follaje en *Solanum tuberosum* L. Rev. Fac. Agron. XLVII: 65–73.
8. GARCIA-TOORES, L. AND C. GOMEZ-CAMPO. 1973. *In vitro* tuberization of potato sprouts as affected by ethrel and gibberellic acid. Potato Res. 16: 73–79.
9. GOTO, N. AND Y. ESASHI. 1974. Differential hormone responses in different growing zones of the bean hypocotyl. Planta 116: 225–241.
10. HAWKER, J. S., J. L. OZBUN, AND J. PREISS. 1972. Unprimed starch synthesis by soluble ADP-glucose-starch glucosyltransferase from potato tubers. Phytochemistry 11: 1287–1293.
11. KANG, B. G. AND S. P. BURG. 1973. Influence of ethylene on nucleic acid synthesis in etiolated *Pisum sativum*. Plant Cell Physiol. 14: 981–988.
12. LANGILLE, A. R. 1972. Effects of (2-chloroethyl)phosphonic acid on rhizome and tuber formation in the potato, *Solanum tuberosum*. J. Am. Soc. Hort. Sci. 97: 305–308.
13. LAWRENCE, C. H. AND W. G. BARKER. 1963. A study of tuberization in the potato *Solanum tuberosum*. Am. Potato J. 40: 349–356.
14. MADEC, P. AND P. PERENNEC. 1962. Les relations entre l'induction de la tubérisation et la croissance chez la plante de Pomme de terre (*Solanum tuberosum* L.). Ann. Physiol. Vég. 4: 5–84.
15. MEHARD, C. W., J. M. LYONS, AND J. KUMAMOTO. 1970. Utilization of model membranes in a test for the mechanism of ethylene action. J. Membrane Biol. 3: 173–179.
16. MINGO-CASTEL, A. M., F. B. NEGM, AND O. E. SMITH. 1974. Effect of carbon dioxide and ethylene on tuberization of isolated potato stolons cultured *in vitro*. Plant Physiol. 53: 789–801.
17. MITZ, M. A. 1974. Primitive control of cellular metabolism. *In:* K. Dose, S. W. Fox, G. A. Deborin, and T. E. Pavlovskaya, eds., The Origin of Life and Evolutionary Biochemistry. Plenum Press, New York. pp. 331–338.
18. NELSON, R. C. 1939. Studies on production of ethylene in the ripening process in apple and banana. Food Res. 4: 173–190.
19. RIDGE, I. 1973. The control of cell shape and rate of cell expansion by ethylene: effects on microfibril orientation and cell wall extensibility in etiolated peas. Acta Bot. Néerl. 22: 144–158.
20. SINGH, G. 1970. Influence of ethrel on growth and yield of potatoes. Res. Life Sci. 18: 38–43.
21. TRIBIS, V. P. AND V. N. PYATNITSKII. 1974. Carbon dioxide level in soil air and yield of plants. Vestsi Akad. Navuk Belarus. SSR, Ser. Sel'skagaspad. Navuk 1: 40–42.
22. YAMAGUCHI, M., W. J. FLOCKER, F. D. HOWARD, AND H. TIMM. 1964. Changes in carbon dioxide levels with moisture in fallow and cropped soils and susceptibility of potatoes to Black Spot. Proc. Am. Soc. Hort. Sci. 85: 446–456.