A RELATION BETWEEN ETHYLENE EVOLUTION AND SPROUTING OF POTATO TUBER

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INTRODUCTION

Since a first approach concerning ethylene evolution of potato tubers was made by detecting the ethylene in the atmosphere above potato stores, a number of investigations on this line have been reported (MEIGH 1959; BURTON 1963; POAPST et al. 1968; PRATT and GOESCHL 1969; BURTON and MEIGH 1971). The studies of ethylene production in plants are greatly facilitated by using gas liquid chromatography. These results identified numerous products including ethylene which were emanated by the potato tubers (POAPST et al. 1968; BURTON 1963). There has been a tendency in the past to consider ethylene as an inhibitor of sprouting involved with potato dormancy (PRATT and GOESCHL 1969). However, a recent investigation revealed that ethylene may be produced in quite small amount from stored potato tubers, insufficient to be an active factor for sprout inhibition of them (BURTON and MEIGH 1971).

On the other hand, ethylene is well known to be a plant hormone initiating fruit ripening and regulating many aspects of plant growth, and there has been accumulated a tremendous amount of information regarding the influence of growth substances on ethylene production of several plants (PRATT and GOESCHL 1969). In the case of potato tubers, the ethylene evolution was also stimulated with gibberellic acid treatment which is one of the most effective agents for breaking their dormancy (POAPST et al. 1968; RAPPAPORT et al. 1957). No available information has been found which directly relates ethylene production and outgrowth of buds in the potato tubers, especially in the excised tissues, although the possible involvement of ethylene in regulation of the tuber sprouting under the bulk storage condition has been discussed by BURTON and MEIGH (1971). A study of this subject with the aim of elucidating a relation between ethylene production of different tissues of the tuber and hormonal treatments of these tissues is of interest to understand the growth of potato sprout.

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MATERIALS AND METHODS

Potato tubers (*Solanum tuberosum* L. cv Irish Cobbler) were used for materials which had been stored in a cellar at 3°C after harvest. Tubers in weight about 30 g were used for ethylene determination of intact tubers and about 100 g for that of excised tissues.

Tissue slice:

Cylinders of tuber tissue were obtained with cork-borer (20 mm in diameter) transverse to axis of the tuber. Cortex and pith of the cylinders were cut transversely into disk-shaped slices (20 mm in diameter and 2 mm in thickness). Similar shaped slices were also obtained from apical part of the tuber including eye. These tissues were divided into three groups depending on the part from where they were derived. Each group of them was used for determining ethylene evolution.

Incubation for ethylene detection:

Ten slices of tissue belonged to each group were placed in 300 mℓ Erlenmeyer flask contained with 5 mℓ of 1 mM phosphate buffer at pH 6.8 with or without growth regulators. All growth regulators were prepared in 10^{-3} M phosphate buffer pH 6.8 and adjusted with 0.1 N NaOH to pH 6.8 when necessary (MAPSON and WARDALE 1966). The buffer was used for control. Penicillin G at a final concentration of 0.25 mM served as the antibiotic during the incubation period. The flask was sealed with two holed rubber stopper in which fitted with air inlet and exhaust port. In all experiments these flasks were held for 20 hr in dark at 25°C with continuous supply of air as described below. In the case of intact tuber, 800 mℓ wide mouth flasks were used for container in which 6 tubers were placed, then they were incubated as the same condition as the tissue slice experiment.

Air supply system:

Accumulation of carbon dioxide in the incubation container resulting from the respiration of potato may be anticipated to affect the physiological efficiency of ethylene (ABELES 1973). To circumvent their problem, fresh air should be constantly supplied to the container (Figure 1). Therefore fresh air drown from outdoor by pump was passed through successively three columns of a tightly packed absorbent cotton, an active charcoal and a $KMnO_4$ absorbed silica gel, in order to minimize a contaminated ethylene and other fumes in the air stream (ABELES 1971). The air was subse-

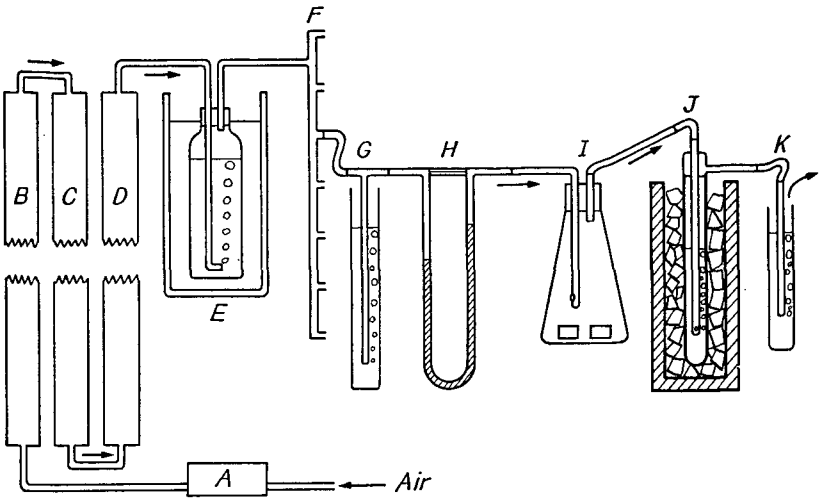

Figure 1. Trapping apparatus of ethylene evolvted by potato tubers and tissues. A=air pressure pump. B=column tightly packed with absorbent cotton. C=column filled with active charcoal. D=column filled wih $KMnO_4$ absorbed silica gel. E=bubbling tower maintained at 25°C water bath. F=manifold. G=barostat tower. H=air flow meter. I=container. J=ethylene trapping tube with mercuric perchlorate solution maintained at 0°C by ice. K=back pressure tower. Spiral glass in the water bath as described in the methods is omitted here.

quently circulated through the spiral glass coil from a large temperature controlled bath to maintain at 25°C. Afterward, stable and accurate regulation of main air stream is accomplished by use of a barostat tower, and the air is humidified by bubbling through water. Finally air stream was divided into the seven containers passed through a manifold, and each constant stream of air was led over the tissues or tubers in the incubation container at a rate of 20 mℓ per minute. The air stream with evacuated ethylene from the container was washed with a solution of mercuric perchlorate which was retained at 0°C. The accumulated ethylene was later released from the complex by the addition of 2 N HCI, and it is measured by gas liquid chromatography (YOUNG et al. 1952).

Gas liquid chromatography:

Ethylene was measured by gas liquid chromatography equipped with a stainless column, 75 cm long and 3 mm in diameter, containing activated alumina (60–80 mesh) and flame ionization detector.

Unless otherwise indicated. at least three replications were run for each experiment. Additional details of experimental methods are described in the text and in the legends of tables and figures where applicable.

RESULTS

Ethylene evolution of intact potato tubers:

Potato tubers stored under the cold condition remained to suppress sprouting throughout most of the storage period. There was a slight evolution of ethylene from the tubers immediately after removal from the cold storage room. The ethylene evolution of the tubers became to increase in time with starting to sprout, if they were allowed to stand in the dark room at about 25°C (Table 1). Accordingly, the increase in the ethylene production seems to be due to onset of the sprouting. On the other hand, greened tubers being exposed to diffused light also produced more amount of ethylene compared to the slight sprouting tubers placed in the light room (Table 1). However senile tubers having been placed in the dark room at about 25°C for long duration emanated less amount of ethylene based on per tuber than that of young ones (Table 2). As comparing the ethylene evolution of them based on per fresh weight of tuber, however, the amount of ethylene from the senile tubers was somewhat higher than that from young ones, because of dehydration of the senile tubers leading to a marked decline in the fresh weight.

TABLE 1. Change in ethylene evolution of potato tubers placed under various conditions

Condition	Amount of evolved ethylene	
	nℓ/ hr/ tuber	nℓ/ hr/ fresh wt in Kg
3°C in darkness	0.05	1.45
25°C in darkness		
non-sprouting	0.14	4.12
sprouting	0.52	14.50
25°C in light		
slight sprouting	0.11	3.09
green sprouting	0.44	5.66

In each experiment, six tubers were incubated in darkness at 25°C for 20 hr. The data represented an avarege of the three experiments. Each condition means that the tubers were allowed to stand under condition for 3 weeks.

TABLE 2. Comparison of ethylene evolution between young and senile tubers

Age	Amount of evolved ethylene	
	nℓ/ hr/ tuber	nℓ/ hr/ fresh wt in Kg
Young tubers	2.03	16.70
Senile tubers	1.51	18.62

Young tubers had been maintained at 25°C for 14 days after removal from cold storage room, and senile tubers had been stored at room temperature for 3 months before experiment.

Changes in ethylene evolution of intact tubers treated with growth regulators:

Some growth regulators involved in the development of potato sprouts give attracted considerable attention, yet basic understanding of relation between sprouting and ethylene evolution of potato tubers remained to limit (BADIZADEGAN et al. 1972; MORRIS 1967; OKAZAWA 1967; GUTHRIE 1938; RAPPAPORT 1969). To verify whether the growth regulators produce effect on the ethylene evolution of potato tubers or not, α-naphthaleneacetic acid (NAA), gibberellic acid (GA) and 6-benzyladenine (BA) were used for this experiment. From an inspection of data of Table 3, it is readily seen that all of the regulators caused a significant increase in ethylene evolution of the treated tubers, especially GA enhanced the ethylene evolution by about five-fold amount as well as the stimulation of sprout elongation compared to the control ones. A marked stimulation of the sprout growth was resulted only from GA treatment, while the stimulation was rarely observed

TABLE 3. Effect of NAA, BA and GA on ethylene evolution of potato tubers

Treatment	Number of sprout per tuber	Amount of evolved ethylene nℓ/ hr/ fresh wt in Kg
Control	6.4	1.90
NAA	1.6	2.12
BA	6.8	3.03
GA	9.0	11.38

Tubers were soaked in the following solutions for 24 hr, then 7 days later ethylene evolution was determined. Concentrations of chemicals were 5.4 M of NAA, 1.1×10^{-4} M of BA and 1.5×10^{-4} M of GA, and control was also soaked in water.

in the tubers treated with BA in place of GA. On the contrary, when the tubers were treated with NAA at 5.4 M, an appreciable increase in ethylene evolution also occurred, notwithstanding that no symptons other than inhibition of sprout development were noted. Taking the fact into consideration, therefore, it is still difficult to conclude that there is a direct causal relationship between the ethylene evolution and the sprout growth in potato tubers.

Ethylene evolution of excised potato tissues:

To further elucidate with respect to the ethylene evolution by the three groups of potato tissues derived from the apical part, the cortex and the pith of tubers, the following experiments were performed. By comparing amount of ethylene produced by each group, it appeared to persist a higher level of ethylene evolution by the group derived from apical part of tuber

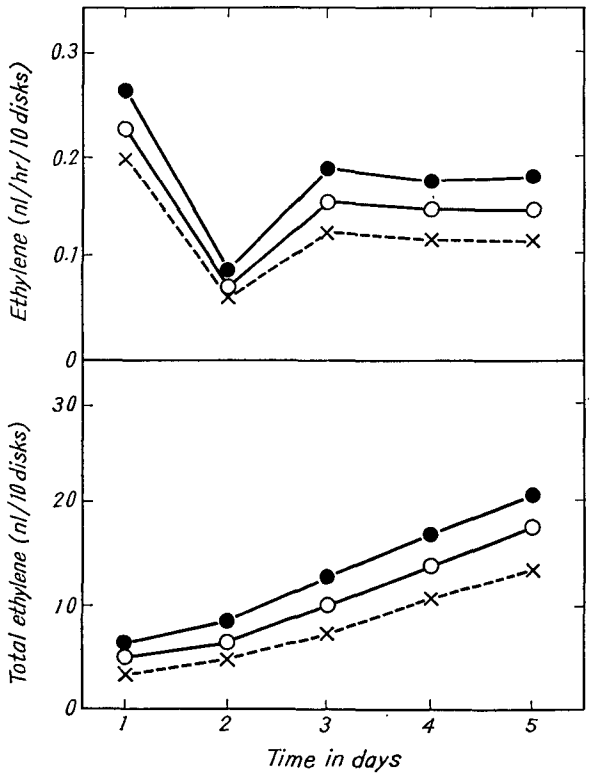

Figure 2. Time course experiment of ethylene evolution of excised tissues derived from various parts of tuber.
—●—=apex, —○—=cortex, --×--=pith.

than that from the other groups throughout the experimental period (Figure 2). On the initial day of this experiment, a high activity of ethylene evolution would presumably be mainly the reflection of wounding effect on the ethylene synthesis of the tissue disks in response to the excision of the tubers (McGLASSON 1969). Afterward the amounts of evolved ethylene in any group remained more or less constant passed through a temporary decline on the second day, so far as concerning this experiment. According to the results that the cortex tissues without eyes or the apical tissues with eyes produced a more amount of ethylene than that by the pith tissues, it can be deduced, therefore, that the peripheral layer of tuber produced ethylene dominantly. From the results showing in Table 4, concern-

TABLE 4. Effects of NAA and GA on ethylene evolution of excised tissues derived from various parts of tuber

Treatment	Part	Amount of evolved ethylene	
		nℓ/hr/10 disks	% of control
Control	Apex	0.18	100
	Crtex	0.15	100
	Pith	0.13	100
GA	Apex	0.30	167
	Cortex	0.18	120
	Pith	0.14	108
NAA	Apex	0.14	78
	Cortex	0.40	267
	Pith	0.34	262

Concentrations of GA and NAA were 10^{-4} M and 1.3×10^{-4} M, respectively.

ing the effectivity of the growth regulators on the ethylene synthesis of the excised tissues, the GA treatment which exerts a promoting effect on elongative growth of sprout took place a marked stimulation of ethylene evolution in the apical tissues of tubers bearing buds, whereas no significant increase in ethylene evolution occurred in the pith tissues of them. This phenomenon may be probably mirrored in the active ethylene synthesis of devoloping buds, and this assumption is also supported by the evidence that there was no appreciable stimulation of ethylene production in the pith tissue of tuber. While the application of NAA at 1.3×10^{-4} M to the apical tissue resulted in an effective retardation of the outgrowth of bud following to somewhat suppress their ethylene evolution, a marked stimulation of the

ethylene evolution in the pith and cortex tissues in the absence of buds was observed. Taking a poor distribution of endogenous auxin in the central pith of tuber into consideration (HEMBERG 1954), it would be inferred that a lack of the effectivity by GA seems to be due to shortage of the endogenous auxin.

In order to clarify this problem by avoiding the effect of the buds on ethylene synthesis, central pith tissues consisted of uniform parenchymatous cells were used. Unlike the results obtained by the cortex tissues or the apical tissues with buds, it is confirmed that BA or GA by itself expectedly exerted no appreciable effect on enhancement of ethylene evolution in the pith tissues (Table 5). In contrast, only NAA was capable of producing a profound improvement of ethylene evolution in the pith tissue, notwithstanding the absence of growing bud which is the site of preferential cell division.

TABLE 5. Effects of NAA, BA and GA on ethylene evolution of pith tissues of tuber

Treatment	Amount of evolved ethylene	
	nℓ/ hr/ 10 disks	% of control
Control	0.96	100
NAA	2.40	229
BA	0.96	100
GA	1.24	130
NAA+BA	2.47	258
NAA+GA	2.79	292
NAA+BA+GA	2.16	225
BA+GA	0.51	53

Concentrations of NAA, BA and GA were 1.3×10^{-4} M, 10^{-4} M and 10^{-4} M, respectively.

DISCUSSION

The present investigation added confirmatory support to the previous conclusion proposed by BURTON and MEIGH (1971) that the stored potato tubers produced ethylene in minute amount. As showing in the Table 1, ethylene produced by the intact tubers was also detected only little amount, if any, at the low temperature, whereas the tubers being placed at the warm condition about 25°C evolved a relative high amount of ethylene. Especially the tubers starting to sprout became to produce much more amount of ethylene with the advance of growth of the sprouts.

PRATT and GOESCHL (1969) proposed that the production of ethylene in or near meristematic tissues suggested a possible role in cell division. EDWARD and MILLER (1972) is of the opinion that endogenous ethylene is a normal product of plant tissues. In the light of above consideration, it is expected that ethylene production of potato tubers may be implicated in the regulation of cell division in the apical meristem of potato sprouts. Although no sure results could be found a causal relationship between sprouting of potato tubers and their ethylene production (BURTON 1963), the present data lend to substantiate the GA-induced ethylene production of sprouting potato tubers. As listed in Table 3, it revealed that the outgrowth of the potato sprout was enhanced by not only placing under the warm condition but GA treatment, leading a marked stimulation of their ethylene evolution. It is still immature to conclude, however, that the enhancement of sprouting due to GA treatment is a sole cause for improvement of ethylene evolution by the potato tubers. Another interesting point arising from this investigation is the fact that an exogenous application of NAA at the relative higher concentration also made ethylene evolution of tubers increase, nevertheless the sprouting of the tubers was almost completely suppressed by the NAA. It has been general recognized that ethylene production of plant is high in tissues containing auxins or these are applied to tissues (MORGAN and HALL 1962, 1964; BURG and BURG 1966). The fact, taking a high level of endogenous auxin in the potato sprouts into account (OKAZAWA 1967), suggests that the presence of auxin in the tissues, even if endogenously or exogenously, may be prerequisite for the stimulation of ethylene evolution in the potato tubers. The above mentioned experimental results using the whole intact tubers cannot explain where part of tuber does involve in ethlyene evolution most significantly. Accordingly it is necessary to re-examine this problem in connection with experiment on ethylene production of the excised tissues obtained from various part of the tuber, In this case, a wound-caused ethylene synthesis cannot be left out of consideration. For instance, MCGLASSON (1969) confirmed that the ethylene production of potato tissue was greatly stimulated by cutting, followed to stabilize at a low rate 24 hours after excision. Therefore ethylene evolution of each tissue was estimated continuously for 5 days, so as to obviate this problem. The results shown in Figure 1 that the cortex tissues or the apical tissues with eyes which obtained from outer peripheral layer of the tuber produced more amount of ethylene compared to that of the central pith of the tuber. The fact is considered to be due mainly though not entirely to the presence of endogenous auxin in the cor-

tex or apical tissues, citing an evidence that there is a substantial amount of auxin in the peripheral tissue of the tubers (HEMBERG 1954). Contrarly, the central pith tissue of tuber was incapable of increasing in ethylene evolution significantly, unless the tissue was fed NAA. Substitution of BA or GA for NAA failed to stimulate ethylene formation of the same tissue, unlike the case of intact tubers. It can be deduced, therefore, that the effect of these growth subatances on the excised tissues is somewhat different, in spite of the similarity in the pattern of stimulation of ethylene evolution in intact tubers. This assumption is much strengthened by the results presented in Table 5, which revealed that both GA and BA intensified the effect to certain extent only when these are existed together with NAA. Accordingly, the failure of GA- and BA-stimulated ethylene production in the pith tissues may be interpreted owing to a shortage of the endogenous auxin. Indeed, there is no or little level of endogenous auxin in the pith tissue of potato tuber (HEMBERG 1954). There is mounting evidence to support the idea that BA or GA by itself has no significant effect on ethylene production of some other plant tissues (FUCHS and LIEBERMAN 1968 ; GAMBORG and LA RUE 1971). In conclusion, it may be at least with certainty that the endogenous ethylene production increases with the outgrowth of buds, and auxin is indispensable to stimulate the additional ethylene production of both intact tuber and excised tissue, evenwhen in the presence of cytokinin or gibberellin.

SUMMARY

The present investigation is concerned with ethylene evolution of potato intact tubers and their excised tissues. The intact tubers produced minute amount of ethylene at the low temperature, but its evolution became to increase by removing to warm condition. While the evolution was stimulated with GA or BA treatment enhancing to sprout the tubers, it was also stimulated by NAA treatment at a relative higher concentration which inhibited their sprouting. Using the excised tissue in place of intact tubers, much amount of ethylene evolution may probably mainly originated from the periphery tissues, and BA or GA by itself had no or little effect on the ethylene evolution by pith tissues, unless NAA was simultaneously existed in the tissues. Therefore, it is concluded that auxin is found to be indispensable for ethylene evolution by not only intact tuber but excised tissues.

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