

Effects of Ethylene on Potato Tuber Respiration¹

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ABSTRACT

Treatment of potato tubers (*Solanum tuberosum* L.) with ethylene gas causes a rapid rise in their respiration rate, reaching 5 to 10 times the rate of untreated tubers over 30 hours of treatment and then falling slowly. The response shows a lag of 8 hours, and more than 24 hours of exposure is required for maximum effect; the temperature optimum is near 25 C. In sensitivity to low concentrations and dependence on temperature, the phenomenon is similar to the effect of ethylene on the respiration of climacteric and nonclimacteric fruits. Treated potato tubers returned to air recover their sensitivity to ethylene more slowly than do nonclimacteric fruits (e.g., mature green oranges). It is proposed that the respiratory rise characteristic of ripening in climacteric fruits and of the wound response in plant tissues is induced by a rise in endogenous tissue ethylene.

Ethylene is a plant hormone having diverse effects on a wide range of plant tissues. In spite of its apparent importance as a regulator of plant growth, the site and mechanisms of its actions have not yet been elucidated (10, 12). We recently described the remarkable effect of this substance on the respiration of intact potato tubers (14) and suggested the possible value of the phenomenon as a simple system for the study of the mode of action of ethylene. We report here a more detailed study of the respiratory response of potato tubers to ethylene gas and discuss the mechanisms that may be involved.

MATERIALS AND METHODS

Mature (100 day) potato tubers (*Solanum tuberosum* L., cv. White Rose) were dug from the field, washed, and stored at 20 C until needed; all experiments except the temperature trial were conducted at this temperature. Commercially obtained tubers were found to be unsatisfactory because previous cold storage and careless handling may have had pronounced effects on their physiology. Mature green sweet oranges (*Citrus sinensis* Osbeck, seedling) were obtained from a local garden.

Respiration measurements were made by placing individual specimens in 500-ml jars ventilated with a stream of air flowing at about 1.5 liter per hr and measuring the CO₂ content of the effluent air by gas chromatography. Ethylene was in-

roduced into gas streams from a pressure cylinder through a reduction valve and appropriate capillary flowmeters, and the final concentration was verified with a gas chromatograph.

When it was necessary to measure respiration in a static system, individual specimens were placed in 9-liter jars, and ethylene in appropriate amounts was added through a rubber septum. Samples of the jar atmosphere were taken through the septum for determination of the CO₂ content. Changes in concentration resulting from sample removal were insignificant, and the jar volume was such that the concentration of CO₂ seldom rose above 0.5%.

RESULTS

Effect of Ethylene Treatment on the Respiration of Potato Tubers. The respiration pattern of potato tubers treated for different lengths of time with 10 μ l/l ethylene is shown in Figure 1. After a time lag of about 8 hr, the respiration rate of treated tubers increased rapidly, reaching a peak 25 to 30 hr after the start of the experiment. The peak rate shown by continuously treated tubers was about eight times the initial respiration rate, whereas that of untreated tubers showed little or no change. Short treatment times were insufficient to bring about such a large effect, although treatment for as little as 3 hr induced a slight increase in respiration over that of the control tubers. The full effect of ethylene was seen only in tubers treated for more than 24 hr.

Effect of Ethylene Concentration. The respiration of potato tubers under continuous treatment in a static system at different concentrations of ethylene is shown in Figure 2. Partial stimulation of respiration was observed in potatoes treated with 0.15 μ l/l, and full stimulation was observed with any concentration above 2.0 μ l/l. The respiration maximum occurred at the same time for all concentrations of ethylene.

Effect of Temperature. The respiration of potato tubers in static systems containing 10 μ l/l ethylene at different temperatures is shown in Figure 3. Temperature had a striking effect on both the magnitude and the timing of the respiratory rise. The peak respiration increased with temperature to 25 C but was lower at temperatures above 25 C. The interval between the start of treatment and the respiration peak was reduced by increasing temperature. The respiration of tubers treated at 35 C did not fall markedly following the respiration peak.

Effect of Repeated Treatments. The respiration response of potato tubers to alternate treatments with air and ethylene is compared with the climacteric-like induced response of oranges in Figure 4. The initial response was parallel for both tissues, but subsequent treatments had little effect on the respiration of potato tubers, whereas oranges responded to each reapplication of ethylene. The respiration rate of oranges was higher than that of potatoes at all times.

Recovery of Tubers from an Initial Ethylene Treatment. The respiration rate of a large lot of potatoes treated with ethylene for 1 day and then returned to air is shown in Figure

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5. The respiration rate of the treated tubers returned to that of the control by about 4 to 5 days after the removal of ethylene. The respiration rate of individual tubers taken at intervals from the above lot and retreated with ethylene is shown also. Renewed application of ethylene slightly stimulated respiration of once-treated tubers as little as 3 days after cessation of the initial treatment. The respiration response to the second treatment gradually increased with extension of the recovery period, reaching about 70% of the initial response 19 days after termination of the first treatment.

DISCUSSION

Increasing implication of ethylene as a major hormone in plants has led to an intensified effort to elucidate the mode

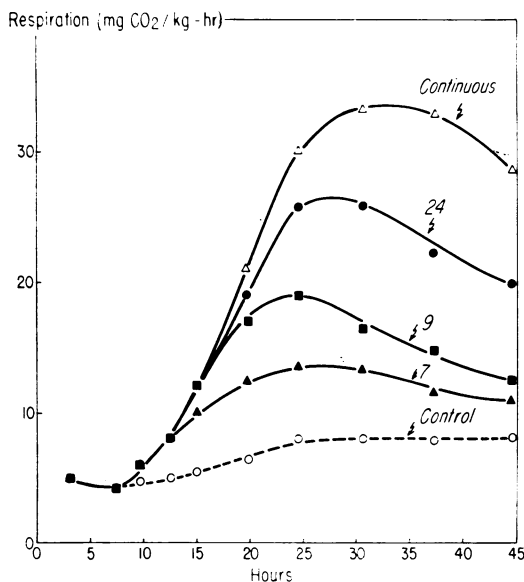


FIG. 1. Respiration of single potato tubers treated from zero time with 10 µl/l of ethylene for various numbers of hours at 20 C. Four other treatment times gave expected intermediate results, but the curves have been omitted for clarity.

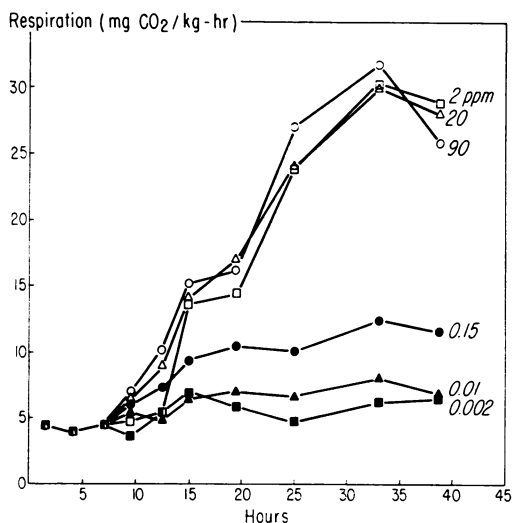


FIG. 2. Respiration of single potato tubers treated continuously with differing concentrations of ethylene at 20 C. The treatment "0.002 µl/l" is the untreated control but reflects air pollution plus endogenous ethylene.

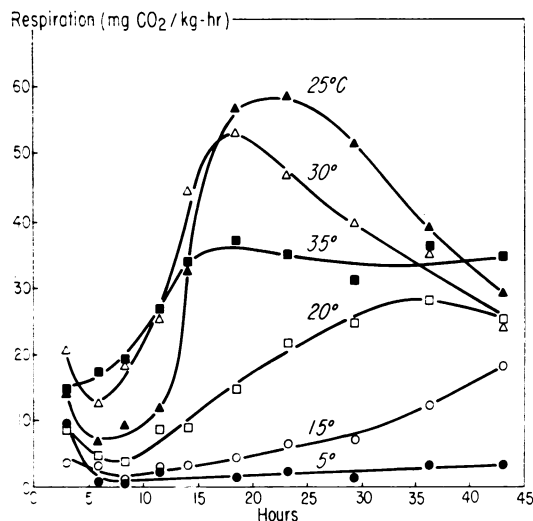


FIG. 3. Response of single potato tubers to 10 µl/l ethylene at different temperatures.

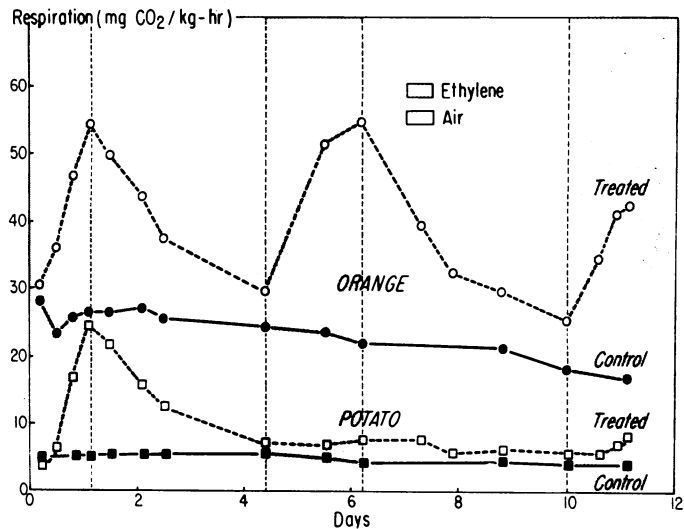


FIG. 4. Response of potato tubers and mature green orange fruits to alternate atmospheres of air and 10 µl/l ethylene at 20 C; the ethylene treatment was applied during the times indicated by shading. Points for the treated specimens represent the mean of four individuals; for the control specimens, they represent the mean of two.

of action of the gas. The respiratory response of resting potato tubers seems markedly simpler than many of the other systems that have been studied, such as ripening fruits and germinating seeds (12), in which profound metabolic and morphological changes are under way, many of them presumably without any direct relationship to the action of ethylene.

The view that resting potato tubers are typical of tissues whose respiration is insensitive to ethylene (19) stems from the work of Huelin and Barker (7); they found relatively small increments in the respiration of treated tubers, probably because of the lower temperature of their experiments, and possibly because of unknown factors in the previous handling of their material. We found the effect of ethylene on the respiration of carefully handled potato tubers to be dramatic, the respiration increasing 8- to 10-fold over a 30-hr period at moderate temperatures.

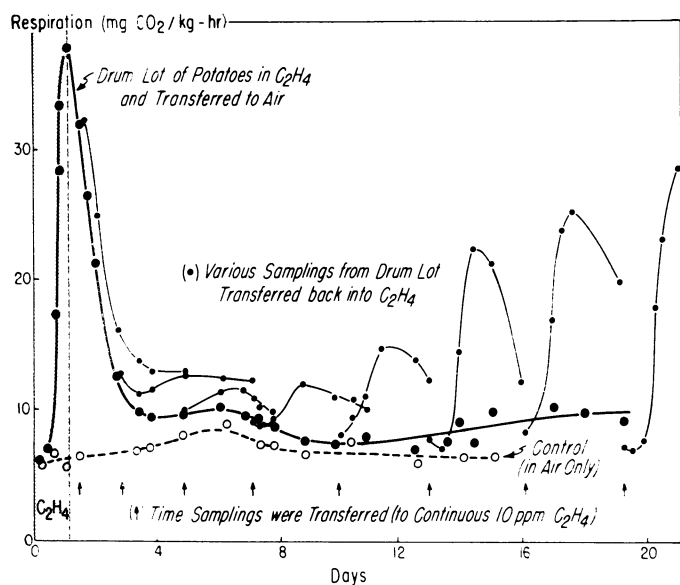


FIG. 5. Recovery of the ability of potato tubers to respond to treatment with $10 \mu\text{l/l}$ ethylene at 20°C .

Treatment of tubers with ethylene for different lengths of time resulted in a proportional stimulation of the final respiration. Quite short treatments were sufficient to elicit a marked rise but in all cases there was an initial lag of at least 8 hr before the commencement of the respiratory rise. Since it has been demonstrated (3) that the gas exchange of potato tubers is sufficiently free to allow rapid equilibration of the interior of the tissue with the external atmosphere, this lag would not seem merely to be time for thorough diffusion of ethylene into the tubers. It is therefore unlikely that the respiration increase is due to a simple physical effect of ethylene on gaseous exchange. Rather, the lag phase resembles the latent period required for the initiation of protein syntheses associated with other inductive events in plants. The fact that a short treatment with ethylene will lead to a later partial rise in respiration also indicates that the lag phase is an active period during which the mechanism of the subsequent respiratory rise is set in motion. Some support for this hypothesis comes from the observations of Stahmann *et al.* (17), who showed increased enzyme levels in sweet potato tissue pieces treated with ethylene, and Shannon *et al.* (16), who demonstrated that this increase involves *de novo* protein synthesis.

The shape of the actual respiration curve suggests that, following the lag period, there is an ethylene-induced but respiration-linked formation of some product which causes the eventual decrease in respiration when the product "feeds back" on its own synthesis. Huelin and Barker (7) showed increased levels of reducing sugar in ethylene-treated potato tubers. It could be that ethylene treatment causes an increase in the rate of starch hydrolysis or other glycolytic events, leading to an increase in the levels of high energy and reduced intermediates, and that these intermediates feed-back on the respiration mechanism. Alternatively, there may be a requirement for a preformed substrate, and respiration decreases when the substrate is depleted. These hypotheses are supported by the apparent insensitivity of treated tubers to a closely subsequent ethylene treatment. In a resting tuber, the metabolic rate is so low that one could infer that the above postulated product or required substrate would be used or reformed slowly, thus explaining the long period required for recovery of the response to ethylene. The effect of repeated treatment of oranges (Fig. 4) supports this suggestion, since

one would expect the critical metabolic pool to be used up (or re-formed) much more rapidly by the higher basal metabolism of these fruits, resulting in their earlier recovery of sensitivity. In climacteric fruits, on the other hand, endogenous ethylene keeps the respiration rate high until the fruits are fully into a senescent phase. The recovery pattern is not seen because the fruits disintegrate.

These considerations lead us to suggest that the mechanism of this respiratory response involves an initial event or process stimulated by ethylene, during which the capacity for protein synthesis, perhaps of those proteins responsible for increasing the rate of glycolysis, is "turned on". In the absence of any sink for the energy intermediates thus synthesized in an intact tuber, they eventually suppress their own synthesis, resulting in the observed respiratory curve and the prolonged insensitivity of the tissue to further treatments with ethylene.

The physiological observations reported here appear pertinent to studies on the role of ethylene in the climacteric rise in fruit respiration and in the phenomena associated with injury in plants such as the induced respiration of tissue slices. Based on the shape and timing of the respiratory curve which follows ethylene treatment of tissues, we have already suggested (14) that the climacteric rise in fruit respiration may be a respiratory response to ethylene common to many parenchymatous tissues in the presence of ethylene. The more detailed studies of the potato response reported here give further support to this hypothesis. The effect of temperature on the respiratory response of potato tubers under the influence of ethylene (Fig. 3) is strikingly similar to that reported by Biale and Young (1) for the respiration of ripening avocados, with a sharp optimum at 25°C and a reduced and modified response at higher temperatures. The concentration of ethylene required to cause a maximal response is very close to that required to stimulate fruit ripening (2, 11). It is clear that fruit ripening is a complex of several processes which may be unrelated to one another once they have been initiated by ethylene in its role as the fruit ripening hormone (11, 12, 15). Although it has been thought that the respiratory increase in ripening fruits might be regarded as the summation of the energy requirements of other events in the ripening complex, recent work (5, 6, 18) has shown that various events of normal ripening can occur in fruits independently of effects on the respiration pattern. Like Quazi and Freebairn (13), we feel that the respiratory climacteric is not the primary biochemical event of ripening; instead it is a parallel event, induced by endogenous ethylene, but still a useful index to the state of ripening of climacteric fruits. Nonclimacteric fruits are probably those that do not show an autocatalytic production of ethylene and consequent respiratory rise as part of their ripening behaviour (4, 14).

It is well known that plant tissues produce abundant ethylene when damaged (12). Subsequently the respiration of the tissue increases, a phenomenon dubbed the wound response. When discs are cut from potato tubers, the respiration rate of these slices immediately increases to three to five times that of the intact tuber (8). During the subsequent 24 hr, the respiration increases further by a factor of three to five. The physiology of this latter response has been much studied, and the mechanism of the increase is still the subject of controversy. Laties (8) has argued, on the basis of respiration rates of slices of different thickness, that a volatile inhibitor present in the tissue diffuses from freshly cut discs. It has been shown that potato slices, like many other injured plant tissues, produce significantly increased amounts of ethylene for 12 hr after cutting (9), and it is reasonable to postulate that the subsequent respiration increase may be induced by this endogenous ethylene. The data which led Laties (8) to postulate a

volatile inhibitor could equally well be used to argue for a volatile activator (*i.e.*, ethylene) diffusing inward from the damaged surface cells.

Other evidence argues a role for ethylene in wound responses, notably the work of Stahmann *et al.* (17), showing effects in sweet potatoes which occur either when the root is damaged or when intact roots are treated with ethylene. The changes so induced enable the tissue to resist infection by pathogens. It is therefore possible that in this situation ethylene may be a "wound hormone;" the initial response of tissues to damage is production of ethylene which in turn induces the wound respiration and associated processes of repair. Thus the respiratory response of potato tubers to ethylene may well represent a normal physiological response of the tissue to a supposed injury signalled by ethylene treatment. The elucidation of the signal mechanism in this comparatively simple ethylene response may well be a useful tool for investigating the control that ethylene exercises in plant tissues.

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