THESIS

ETHYLENE PRODUCTION BY THE POTATO TUBER

Submitted by

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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY DAVID LEE CREECH ENTITLED ETHYLENE PRODUCTION BY THE POTATO TUBER BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

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ABSTRACT OF THESIS

ETHYLENE PRODUCTION BY THE POTATO TUBER

Russet Burbank tubers were stored at 32°F and 45°F and continuously ventilated with atmospheres of 2% O_2, air, 80% O_2, 4% CO_2, 12% CO_2, and intermittently ventilated with air. Ethylene production by the tubers was traced throughout a seven month storage period. Tubers stored in atmospheres of 2% O_2, air, 4% CO_2, and intermittent air at 32°F and 45°F evolved ethylene at a rate no greater than 0.008 ul kg^{-1} hr^{-1} throughout the storage period. In all cases where sprouting occurred, the rate of ethylene production increased. Tubers stored in 80% O_2 and 12% CO_2 succumbed to physiological breakdown and produced ethylene at rates much greater than the rates for tubers stored in the non-toxic atmospheres (2% O_2, air, 4% CO_2, and intermittent air). The peak rate of ethylene production observed was 0.300 ul kg^{-1} hr^{-1} for tubers stored in 80% O_2 at 45°F. In general, the higher temperature produced the higher rates of ethylene production. The only exception to this rule were those tubers stored in 12% CO_2.

Tubers inoculated with Alternaria solani and Fusarium roseum var. sambucinum were investigated for the ethylene-producing ability. Uninoculated tubers and tubers inoculated with Alternaria solani

evolved ethylene at approximately the same rate. Tubers inoculated with Fusarium roseum, however, demonstrated greatly stimulated ethylene production, often as high as 0.100 ul kg^{-1} hr^{-1}. Cultures of Fusarium roseum grown on PDA failed to produce ethylene.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

I. INTRODUCTION

The potato tuber in storage is often subjected to the extremes of temperature, atmosphere, humidity, and pressure. The harvesting, handling, and loading of potato tubers into bins encourages the invasion of pathogens. Infection 'hot spots' within the bin sometimes cause the tubers to melt away as the disease progresses. In the case of stored seed potatoes, the productivity of future crops is affected by the entire gamut of storage conditions.

The role of volatiles, such as ethylene, as a storage factor is still unknown. Potatoes produce relatively little ethylene but it has been observed to accumulate in poorly ventilated storages. In high concentrations ethylene exerts its effect on the rate of respiration, on sprouting, and on many metabolic centers within the tuber itself.

The advent of the new line of sophisticated gas chromatographs has pushed the state of the art to a point that researchers can now detect and measure ethylene concentrations in the low ppb range. Partly as a result of this, the volume of ethylene research has reached tremendous proportions and numerous reviews have been compiled from the literature (8, 34, 46).

The purpose of this work was to analyze the effects of various storage conditions on endogenous ethylene production by potato tubers. An understanding of the tuber's capacity to evolve ethylene at any

point in its storage period is essential if the role of this volatile is to be understood in its entirety.

Potato tubers are often subject to the ravages of fungal and bacterial organisms while in storage. Many pathogens have demonstrated the ability to evolve ethylene at high rates. Two common storage pathogens, Fusarium roseum var. sambucinum and Alternaria solani, were investigated for their ethylene-producing ability.

II. LITERATURE REVIEW

The purpose of this survey is to review past work related to the production of ethylene by potato tubers and its effects on tuber tissue. The role of wounding and disease in ethylene production is also considered.

Effects of Ethylene

The effects of ethylene on the dormancy of the potato tuber were obscurely recognized long ago but are clouded in the literature with references to the emanations of "growth-inhibiting substances" by fruit tissues. A considerable amount of work was directed toward controlling the rest period of potato tubers. Rosa was prompted in 1923 to test a tremendous number of chemicals for their ability to break the dormancy of resting tubers. Dipping cut seed pieces in a 0.5 Molar solution of $NaNO_3$ resulted in shorter sprouting times and higher germination percentages (50).

An ethylene derivative, ethylene chlorhydrin, received some early acclaim as a terminator of rest in dormant potato tubers. Denny in 1926 tested 224 chemicals for their ability to break the dormancy of potato tubers and found two that were effective: ethylene chlorhydrin and thiocyanate (16). Loomis in 1927 found that seed tubers treated with ethylene chlorhydrin had more total weight of

sprouts and shorter emergence times than untreated tubers although germination percentages were slightly less (35). Stuart and Milstead observed that ethylene chlorhydrin broke the rest period of potato tubers and produced growth in ten to twenty days (61). Ethylene chlorhydrin terminated the rest of two Indian potato varieties, 'Phulwa' and 'Qualmi', when applied at the rate of 20cc ethylene chlorhydrin per 100 grams of tuber tissue (44). Michener hypothesized in 1942 that the disappearance of apical dominance and the encouragement of sprouting following ethylene chlorhydrin treatments were the result of auxin destruction (40). Rappaport et. al. in 1968 reported that ethylene chlorhydrin treatments greatly encouraged the elongation of buds and mitotic activity in potato tubers (cited in 44).

The effect of ethylene gas on the dormancy of potato tubers is somewhat confused by the appearance of contradictory reports in the literature. Vacha in 1927 subjected potato tubers to high concentrations of ethylene (1:1000) only three times at 36 hour intervals and observed shorter sprouting times and faster growth (63). Rosa tested the effects of ethylene gas on three varieties of potato tubers: White Rose, Idaho Rural, and Irish Cobbler. Ethylene concentrations of 1:5000, and 1:100,000 were examined for their effect on sprouting. Tubers were dug June 24th, exposed to the treatments for one month and then planted. In all cases, the ethylene gas treatments not only increased the emergence rate but also the average number of stems

per plant. The percentage emerged one month after planting for the 1:5000 ethylene treatment for all three varieties was 59% vs. 18% for the Check; the average number of stems for the 1:5000 treatment was 1.48 vs. 1.11 for the Check. The results so impressed Rosa that he outlined the implementation of ethylene gas treatments on a large scale by potato growers (51). Denny observed, however, that ethylene gas treatments in concentrations varying from 75% to 1:5, 000, 000 for periods varying from one hour to seven days were not as effective in stimulating sprout growth as some of the other chemicals tested (16).

Elmer observed the effects of growth-inhibiting substances from several varieties of apples. Potted germinating seed pieces held in the same room with ripe apples failed to develop normally. Potted non-germinating seed pieces held in the same room with ripe apples lost apical bud dominance and abortive multiple sprouting occurred. Six varieties of potatoes were tested and consistent inhibition of sprouting occurred from the emanations of four varieties of apples. No growth inhibition was obtained from the volatiles produced by oranges, immature apples, decayed apples, apple oil, and, interestingly enough, bananas. Keiffer pears gave results similar to those produced by ripe apples (20).

Some stimulation of sprouting was observed by Huelin in 1933 by the injection of ethylene into the storage atmosphere surrounding

different lots of potatoes. His three treatments were: four doses of 12 hr. each week at regular intervals; two doses of 24 hr. each week at regular intervals; one dose of 24 hr. each week. When .1 or .01% by volume ethylene was passed continuously over the tubers in an air stream, Huelin observed the characteristic inhibition of sprouting first described by Elmer. If the per cent of ethylene was reduced to .001% appreciable inhibition of sprouting still occurred (cited in 10). Furlong inhibited the sprouting of 18 tons of potatoes by introducing ethylene gas into the storage bin every third or fourth day. The concentration of ethylene varied considerably but remained higher than 1:10, 000 (cited in 10).

Elmer in 1936 expanded his study on the effects of growth-inhibiting emanations from apples to include some direct observations on the effects of ethylene gas. Potato tubers were stored with apples in boxes from January to early summer at 10°C. The treatments used were: tubers stored with no apples, tubers stored with 1 lb. apples, tubers stored with 5 lb. apples, and tubers stored with 10 lb. apples. Potatoes stored with the 5 and 10 lb. lots of apples exhibited marked sprout inhibition and were of higher quality (very firm and with less decay). Elmer concluded that ten pounds of apples stored with sixty pounds of potatoes provided a sufficient concentration of the growth-inhibiting gas for the maximum prevention of sprouting. Tubers stored with 10 lbs. of apples developed a characteristically sweeter

taste and when planted more stems emerged per plant. Table one illustrates a few of the chemical analyses that were run on treated and untreated tubers and sprouts:

Table 1. Chemical analyses of sprout and tuber tissues from normal potatoes and from potatoes affected by the growth-inhibiting gas from apples (21).

Tissue	Total N %	Total sugars %	Reducing sugars %
Sprout			
normal	3.70	15.40	13.46
abnormal	3.66	20.66	17.41
Tuber			
normal	2.12	0.64	0.0
abnormal	2.11	3.20	0.0

Elmer hypothesized that the growth-inhibiting substance was ethylene and observed that tubers subjected to this volatile (concentration: 1:20, 000) failed to develop normal sprouts. Elmer concluded that the growth-inhibiting substance from apples and ethylene were one and the same (21).

Huelin and Barker observed two distinct effects of ethylene treatment on the metabolism of potato tubers: 1) a rise in the respiration efficiency (respiration rate/total sugars) and 2) a rise in the concentration of sugar. Exposure to ethylene shortly after the tubers were harvested produced only a rise in respiration efficiency and not an increase in sugar concentration. Later in the storage period, however,

both effects were observed. Ethylene did not exert any effects on potato tubers whose sugar content had already been raised to a high level by low temperature storage. High sugar potatoes regained the power to respond to ethylene if the sugar content was decreased by storing at higher temperatures. Huelin hypothesized that the effect of ethylene on the respiration efficiency was directly concerned with the supply of substrate to the respiratory centers. That is, when substrate was high (i.e. high sugar potatoes) ethylene had no effect on respiration. When substrate was low (i.e. low sugar potatoes) ethylene increased respiration efficiency. Huelin felt that the potato tuber's development of sensitivity to ethylene with storage time was the result of a loss of "organization-resistance", a term originally coined in 1928 by Blackman and Parija (5). It referred to a resistance to reaction within tissues because of spatial separations between reactants by protoplasmic membranes. The approach of senescence was accompanied by the degradation of "organization" within the tissue . . . more mixing of reactants and less resistance to reaction. Huelin recognized a basic difference in response to ethylene treatments between fruit and potato tuber tissue. Tubers exposed to ethylene gas responded by increasing rates of respiration; if the ethylene was removed, respiration returned slowly to a normal rate. Fruits, on the other hand, developed irreversible increases in respiration rates after exposure to ethylene. Huelin attributed this to the auto-stimulatory nature of fruit tissue. Fruits subjected to ethylene gas

treatments initiated high endogenous rates of ethylene production while the potato tuber did not (26).

An increased rate of respiration is an almost ubiquitous response to ethylene for morphologically unrelated plant tissues. Ripening, increased rates of respiration, and increased rates of endogenous ethylene production are strongly interrelated processes (38). Potato tuber tissue is morphologically dissimilar from fruit tissue but does respond to ethylene gas in much the same manner. Reid and Pratt in 1970 noted that treatments of potato tuber tissue with ethylene gave "respiration effects similar to those long recognized in fruits" (49).

Inhibited sprouting of potato tubers in storage was often attributed to higher-than-atmospheric concentrations of carbon dioxide. Burton noted, however, that the sprouting of tubers was markedly retarded by scrubbing out CO_2 and leaving the volatiles in the storage atmosphere. Scrubbing out the volatiles and leaving the CO_2 in the storage atmosphere allowed sprouting to proceed normally. Burton concluded that the prolongation of dormancy may be due to ethylene and not the presence of accumulated CO_2 (10).

Burton observed that potatoes in poorly ventilated storage containers accumulated a sufficient concentration of volatiles to markedly inhibit sprouting (12). But, as Burton observed in 1971, ethylene's role in storage atmospheres remains obscure since there

are no indications in the literature that ethylene concentrations below 0.5 ppm would have any effect upon sprout growth (13).

Enzymes often undergo marked changes in activity following ethylene gas treatments. Sweet potato tissue subjected to ethylene demonstrated increased activities of peroxidase and polyphenol oxidase. The increase in peroxidase activity was recognized only 4 hours after ethylene treatment and the addition of CO_2 partly overcame the increase (23, 28, 59). Ethylene treatments of potato tuber tissue produced only an increase in the level of polyphenol oxidase and not peroxidase (59).

Commercial applications for ethylene in the potato growing industry have been few. Initiation and stimulation of adventitious roots were accomplished by exposure of many morphologically unrelated plants to ethylene, acetylene, and propylene. Ethylene was most effective in concentrations varying from .2 to .001% (74). Potato tuber sprouts with 25 cm^3 of mother tissue attached were grown in perlite and placed in boxes in growth rooms. Ethylene was passed through the boxes at a concentration of 50 ppm at a flow rate of 250 ml/minute. In all cases, treatment with ethylene led to an inhibition of sprout and stolon elongation, loss of the positive geotropic response of the shoot, and inhibition of root development. Swellings of all rapidly expanding regions were dissimilar from normal tuber initiations in that they contained little or no starch. Tubers treated

with Ethrel developed conspicuous swellings that were also devoid of starch. It was concluded that ethylene may play a role in tuber initiation and starch deposition does not occur until the tuber reaches a certain size (14).

Amchem Products Incorporated recommend ethrel treatments in the form of dips to initiate more tubers per seed piece. Although tuber size is much reduced, total yields remain the same. Small tubers are often desired in the canning and potato seed industry (cited in 66).

Jackson et. al. observed increased tuber sprouting of 'Yellow Nutsedge' (Cyperus esculentus) that had been treated with Ethrel or ethylene gas. Ethylene was found to be effective in concentrations varying from 3 to 10 ppm in air. CO_2, however, decreased the stimulative effect of Ethrel or ethylene (29). Ethylene inhibited elongation, lateral root initiation, and cambial activity of cultured radish roots. However, 1% CO_2 reversed the effects of ethylene and stimulated root growth. The authors suggested that the often observed stimulation of root growth may be due in fact to an interaction with endogenous ethylene (47).

The role of ethylene in the initiation of tubers is unknown. The ecology of the soil allows waterlogged or compacted systems to build ethylene concentrations of 10-20 ppm (56). The effect such concentrations might have on a field basis is only speculatory.

Ethylene has demonstrated the capacity to alter enzyme activities within potato tuber tissue. Respiration rates and sugar concentrations increase after ethylene gas treatments. Its effect on breaking the rest period of potato tubers is confused by its dual nature; continuous levels of ethylene inhibit sprouting while intermittent ethylene treatments appear to encourage sprouting. Ethylene treatments encourage smaller tubers and more stems per plant. The role of ethylene in the soil and its relationship to tuber initiation remains obscure.

Production of Ethylene

Many plants placed in an atmosphere containing ethylene undergo epinastic responses and some plants drop all their leaves in very low concentrations. Crocker and Zimmerman estimated that the minimum dosage able to cause epinasty in tomato plants was in the range between 0.05 to 0.10 ppm ethylene (15). Denny in 1935 tested many plant tissues for their capacity to produce volatiles that would cause epinasty of potato and tomato plants. Tubers of Irish potatoes, either whole or cut into pieces, were placed in sealed containers for 48 hours. When air surrounding the tubers was transferred to a dessicator containing young potato plants no epinastic response was observed. This relatively simple bioassay was the first proof that potato tubers do not evolve large quantities of ethylene (17).

Burton found that tubers placed in containers that allowed only minimal diffusion of gases exhibited inhibited sprouting because of an accumulation of volatiles. His work led him to believe that during storage ". . . potato tubers evolve volatile substances which will suppress their own sprouting . . . these substances may include both ethylene and the vapour on n-amyl alcohol . . . ." (11). Burton observed that potato tubers evolved volatiles that, if allowed to accumulate, suppressed growth in both 5% CO_2 and 5% O_2 atmospheres (12).

Meigh noted in 1959 that "apples are not unique among plant organs in producing a variety of hydrocarbons. Dormant potatoes have recently been demonstrated to yield a similar range of compounds" (39).

Poapst in 1968 provided the first rigorous chemical proof that potato tubers were capable of producing ethylene. The internal ethylene was evacuated from Kennebec tubers and identified by means of mass spectrometry, and gas and paper chromatography. An internal ethylene content of 0.7×10^{-3} ug g^{-1} of fresh weight was ascribed to tubers that had been stored for seven months (45). McGlasson in 1969 observed that dormant potato tubers evolved ethylene at a rate less than 0.015 ul kg^{-1} hr^{-1} (37).

Any comparison of ethylene production rates of various fruit tissues with potato tubers reveals that the tuber is an almost non-producer. Intact apples evolved ethylene at a rate of 51.6 ul kg^{-1} hr^{-1};

freshly cut mangoes (considered to be a low producer of ethylene) evolved ethylene at harvest at a rate of 4.8 ul kg^{-1} hr^{-1}. The possible contamination of citrus fruits with ethylene-producing fungi has confused the ethylene production picture for these fruits. If citrus fruits are capable of evolving ethylene they do so at a very low rate, 0.02 to 0.06 ul kg^{-1} hr^{-1} (9).

Low oxygen concentrations slowed the rate of color change markedly in green bananas. This was true even if ethylene was supplied to the tissues from an external source (25). Mapson observed that preclimacteric bananas held in low oxygen atmospheres did not evolve ethylene and subsequently did not ripen. If ethylene was added to the low-O_2 environment ripening proceeded normally (38). Apples held in a 2.5% O_2 atmosphere had ethylene production and respiration reduced by more than 50%. Burg noted that ethylene production and oxygen consumption showed an almost identical dependence upon oxygen tension, the half maximum value being reached at 1.5 to 2.0 per cent oxygen (7).

Biale was able to greatly stimulate the production of ethylene by placing Valencia oranges in 100% O_2 (4). Burg observed that very high CO_2 environments (80% CO_2 - 20% O_2) inhibited ethylene formation by 40% in apples. But placing apples stored in this environment back into normal atmospheres (20% O_2 - 80% N) resulted in ethylene production returning to normal rates (7).

The rest period of potato tubers is strongly influenced by hormones (48). The termination of rest in the potato tuber could involve ethylene and gibberellins. Okazawa in 1959 extracted substances from various parts of the potato plant which behaved like gibberellins. These substances were found in the following range of concentrations: sprouts > stem tip > tuber > middle part of stem > foliage (cited in 6). Smith and Rappaport noted that the gibberellin content in potato tubers increased more than thirty fold upon sprouting and reached a final concentration of 3.0 ug kg^{-1} of tissue on a fresh weight basis (54). Poapst in 1968 observed that tubers treated with gibberellin contained significantly higher levels of ethylene (45).

All reports in the literature indicate that potato tuber tissue is a very low producer of ethylene. Burton concluded in 1971 that although the potato tuber did evolve ethylene it was not an important constituent (percentage-wise) of all the volatiles produced (13). The occurrence of sprouting is associated with increasing gibberellin levels. Increasing gibberellin levels have been associated with higher than normal internal concentrations of ethylene. Therefore, sprouting tubers should exhibit an increased level of ethylene production.

Role of Wounding and Disease

Potato tubers responded to slicing, wounding, and bruising by initiating a dramatic increase in the rate of respiration. The

increase depended on the degree of injury, of course, but it was as much as ten fold higher than undisturbed tubers (1, 31, 33).

Increased rates of ethylene production often follow wounding treatments. Cutting of fresh cantaloupes caused an almost immediate increase in the rate of respiration of the slices. Ethylene production by the tissue slices was at least ten times that of the intact fruits (36). Laties in 1962 ruled out the classical explanations for the inverse relationship between thickness and respiratory rate (namely oxygen availability and wounding) and presented the hypothesis that "regulation of respiratory development represents a negative feedback process in which control is effected by a volatile respiratory product". CO_2 was eliminated as a possible volatile to effect such feedback (32). McGlasson demonstrated that freshly cut potato tuber tissue evolved ethylene at relatively high rates (up to 0.4 ul kg^{-1} hr^{-1}) compared to dormant tuber tissue (less than 0.015 ul kg^{-1} hr^{-1}). The high rate of production was reached in the first few hours after cutting and dropped slowly to a low level within 24 hours (37). Imaseki et. al. observed increased rates of ethylene production by sweet potato tissue following chemical as well as physical injury. The rate of ethylene production was proportional to the degree of damage done (28).

The first suggestion that ethylene might be a product of fungal metabolism came with Gane's observation in 1935 that the growth of

pea seedlings was inhibited by the atmospheres surrounding aerobically grown baker's yeast cultures (cited in 8). The response of Alaska pea seedlings to volatile substances was an early and effective bioassay for the detection of ethylene. It has been defined as a change of negative geotropism to diageotropism, increased growth in thickness, and reduced rate of elongation for etiolated Alaska pea seedlings exposed to physiologically active emanations (68).

Hellinga observed that Fusarium spp. and Gibberella, in pure culture as well as on host tissue, produced emanations that stimulated the respiration of potato tuber tissue. However, the evolved substance was thought to be non-volatile which would eliminate ethylene as a possibility (24).

The most extensive investigations of fungal-produced ethylene has been the work with Penicillium digitatum. Miller et. al. observed that volatiles from citrus fruits produced epinasty in potato leaves and citrus fruit inoculated with P. digitatum produced the epinastic response much quicker (41). Biale noticed that P. digitatum was capable of increasing the respiration of lemons (2). The emanations from a single moldy lemon were capable of inducing increased respiration rates and color changes in over 500 fruit. A temperature of 14.5°C was favorable for the maximum production of the respiration-increasing, color-changing volatile while 2.5°C inhibited its evolution. P. digitatum cultures grown on PDA were capable of producing the emanation as well as when grown on fruit (3).

Penicillium digitatum cultures when grown on media provided researchers with an opportunity to study ethylene biosynthesis. Ethylene was identified by Young et. al. as the active emanation from P. digitatum (72). A mutant mold incapable of increasing the respiration of lemons was found to produce significantly less total volatile material (62). Many researchers found that varying the substrates for P. digitatum cultures resulted in different conversion efficiencies to ethylene. The use of this fungal organism as a tool to understand ethylene biosynthesis achieved some acceptance but many problems arose to discount its effectiveness (30, 58, 64, 65). Jacobsen concluded that the ethylene biosynthetic pathway in P. digitatum was distinctly different from the pathway prevalent in plants (30).

Other fungi have been tested for their ability to evolve growth-inhibiting substances. Penicillium expansum was ineffective in stimulating the ripening of Duchess or Wealthy apples (57). The method for that determination was somewhat illusory and this fungus may still be proven to evolve ethylene. Blastomyces dermatitidus, B. brazilienis, and the mycelial stage of Histoplasma capsulatum produced emanations which gave positive responses with etiolated Alaska pea seedlings (43). The emanations of Diplopoda natalensis, Diaporthe citri, and Alternaria citri failed to induce epinastic responses in tomato and potato plants (41).

Rose leaves infected with blackspot evolved considerable quantities of ethylene. If the pathogen was grown on an agar medium

no ethylene was detected by epinastic tests. Cherry leaves infected with shothole, chrysanthemum and carnation flowers infected with ray blight and Botrytis cinera initiated positive responses when tested for ethylene (67). Smith inoculated carnation flowers with Botrytis spp. and observed an earlier rise in ethylene production than uninoculated carnation flowers. Botrytis spp. on a malt agar medium failed to produce ethylene (55).

Williamson in 1950 tested the response of etiolated Alaska pea seedlings to the emanations from diseased and healthy tissues of 12 species of plants. Nineteen pathogens were investigated for their ethylene producing ability and their capacity to stimulate ethylene production. Of five pathogens grown on PDA, only Penicillium digitatum was capable of producing ethylene. Alternaria dianthi stimulated ethylene production on carnation flowers. Phytophthora infestans inoculated onto Green Mountain potato plants stimulated ethylene production slightly over that of the healthy plant (68). The Ray blight fungus (Mycosphaerella ligulicola) on chrysanthemum blooms caused the dropping of snapdragon blooms; this was attributed to ethylene produced by the diseased tissue (19).

The bacterium, Pseudomonas solanacearum, was recognized in 1964 for its prodigious rates of ethylene production and was recommended as an ethylene investigative tool (22).

Dimond in 1953 suggested that Fusarium oxysporum was capable of producing ethylene both on a disease host and in culture. This

organism's ability to produce ethylene in culture depended to a great extent on the type of substrate available (18). Although the validity of this work was questioned by Burg in 1962 (8) several other researchers have reported the ability of Fusarium spp. to evolve ethylene. Kamberbeek observed that Fusarium infected tulip bulbs produced relatively large amounts of ethylene (cited in 42). Tulip bulbs infected with F. oxysporum f. sp. tulipae in the soil soon evolved ethylene at such rates that concentrations rose to above 10 ppm. The ethylene concentration decreased with increasing distance from the infected bulbs. The amount of ethylene in the soil could account for the observed growth inhibition of the shoots and roots and the blasting of flowers of tulips infected with the fungi (42).

Ilag tested 228 species of fungi for ethylene production and found 58 that did evolve ethylene. Alternaria solani was classified as a low rate producer of ethylene. Aspergillus clavatus produced ethylene at the highest rate of all the fungi tested. A survey of 20 unidentified actinomycetes isolated from the soil also showed the presence of ethylene in the atmosphere surrounding some of the cultures. In all cases ethylene was determined by means of gas chromatography (27).

Ross concluded that the emanations from virus infected potato leaves was ethylene. Potato virus Y. (Marmor upsilon H.) symptoms were similar to those of certain plants exposed to ethylene.

Alaska pea seedlings exposed to the emanations of virus-infected tissue gave a positive triple response (52).

Sweet potato tissue infected with Ceratocystis fimbriata produced ethylene at high rates. Interestingly enough, ethylene initiated disease resistance in adjacent tissue. This was explained as an induction of certain enzymes by ethylene in the sweet potato tissue (peroxidase and polyphenol oxidase) that slowed the advance of the pathogen (59).

The effects of ethylene on fungal growth are still largely unknown. What is in the literature does not permit generalization. Ethylene increased the rate of both growth and spreading of cultures of slime molds in all concentrations up to 85% (53). On the other hand, ethylene stimulated the respiration of Aspergillus but retarded its growth and excretion of products into the growing medium (cited in 8).

Conclusions

The potato tuber responds to ethylene treatments by increasing its rate of respiration and sugar content. The activities of certain enzymes have been observed to rise following ethylene treatments. Depending on the length of treatment, ethylene can either inhibit sprouting or encourage it. The effect of very low concentrations is still unknown. Wounded potato tubers evolve ethylene at rates much

higher than that of sound, healthy tissue. The role of fungal organisms in ethylene production by potato tubers in commercial bins may be large. Many fungi have the capacity to stimulate ethylene production on host tissues.

50% COTTON
ENGLISH BOND
BY
FOX RIVER

III. THE EFFECT OF STORAGE TIME, ATMOSPHERES, AND TEMPERATURES ON ENDOGENOUS ETHYLENE PRODUCTION BY POTATO TUBERS

Objective

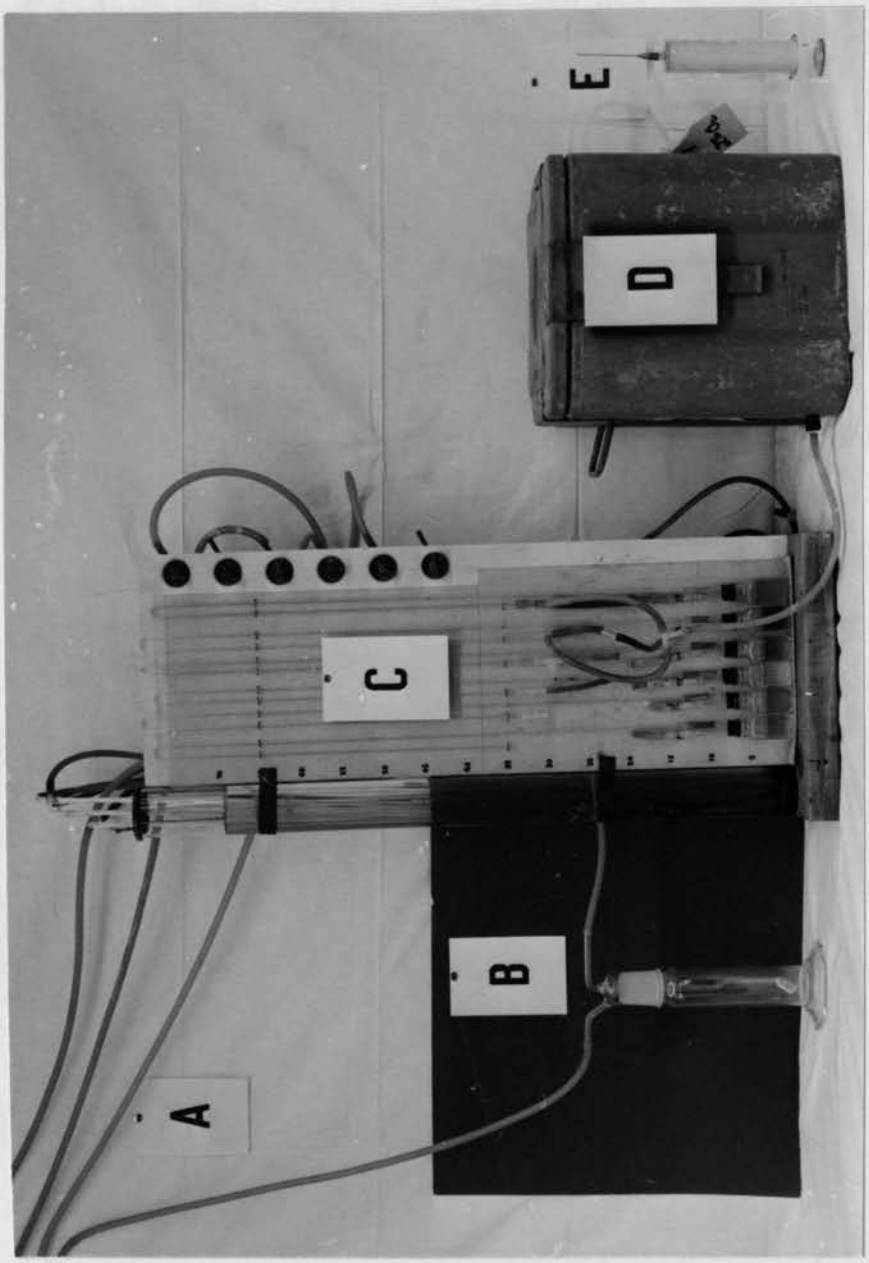
The purpose of this portion of the study was to evaluate the influence of chronological and physiological age as modified by storage conditions on endogenous ethylene production by potato tubers.

Experimental

Russet Burbank tubers grown in the San Luis Valley, Colorado in 1970 were chosen for the study. Foundation seed tubers, 4 to 6 ounces in weight, were selected on a random basis and placed in air tight containers; each container was equipped with an inlet and outlet. The average number of tubers per container was 145 and two containers were joined in series for each treatment. The average weight of tubers per treatment was 36.6 kg.

Two temperatures were selected for the study, 32°F and 45°F. Flowing atmospheres were prepared with flowboards and capillaries using a compressed air source and bottled gases (Fig. 1). Atmospheres were passed through the containers at a flow rate of 8 liters/hour. Atmospheres selected for the study were 2% O_2, air, 80% O_2, 4% CO_2, and 12% CO_2. An additional treatment called intermittent air was

Figure 1. Schematic design of experiment: A) inlets for air and gases B) mercuric perchlorate scrubber for removing background levels of ethylene C) flow board with capillaries for the mixing of gases to create desired atmospheres D) air tight container equipped with inlet and outlet ports E) outlet from which gas samples were collected.


A
B
C
D
E

also included. This involved sealing the containers for 48 hours, flushing with air, sealing for 48 hours, flushing with air, and so on to create a constantly changing status of O_2 and CO_2. The concentration of CO_2 in this treatment varied from 0 to 8% and the level of O_2 varied inversely a corresponding amount. The temperatures and atmospheres selected for the study provided a range of conditions that encompassed those most likely to occur in commercial storages as well as purely theoretical conditions. The influence of these conditions on the physiology and performance of potato tubers was demonstrated by the work of Workman and Twomey (69, 70, 71). Concentrations of O_2 and CO_2 were determined with a Hays-Orsatt gas analysis apparatus to within .2% of the desired level. The experimental design is described in Table 2.

Ethylene was scrubbed from the atmospheres prior to entering the treatment chambers by an ice-cooled mercuric perchlorate solution prepared in the manner of Young et. al. (73). This effectively reduced the background concentration of ethylene entering the containers to less than 1 ppb. One hundred ml gas samples were collected from the outlet ends of the treatment chambers and analyzed for ethylene by gas chromatography. Measurements were taken every three weeks and at least 2 samples were taken per treatment for ethylene analysis.

All ethylene measurements were made in the freeze-out manner of Stephens and Burleson (60). A gas chromatograph fitted with a

Table 2. Experimental design; Russet Burbank tubers grown in the San Luis Valley, Colorado in 1970 were placed into the following treatments after harvest.

Treatment no.	Temperature	Atmosphere	Wt. in kg*
1	32°F	2% O_2	37.0
2	"	Air	35.7
3	"	80% O_2	36.1
4	"	4% CO_2	37.0
5	"	12% CO_2	37.5
6	"	Int. Air**	37.5
7	45°F	2% O_2	36.1
8	"	Air	36.4
9	"	80% O_2	36.4
10	"	4% CO_2	37.0
11	"	12% CO_2	36.1
12	"	Int. Air	36.1

* weight of tubers per treatment, evenly distributed in two containers.

** intermittent air treatment; involved sealing the containers for 48 hours, flushing with air, sealing 48 hours, flushing, and so on to create a constantly changing status of O_2 and CO_2 (CO_2 varied from 0 to 8%; O_2 varied from 21 to 15%).

flame ionization detector (Model 5750, Hewlett-Packard, U.S.A.) provided chromatograms for methane, ethylene, ethane, acetylene, and other assorted hydrocarbons.

Operating conditions were as follows: the column was 1.52 m x 2.38 mm packed with Porapak N (100/120 mesh), oven temperature 60°C, detector temperature 210°C, N carrier gas at a flow rate of 80 ml/minute. A freeze trap (1/8th inch OD stainless steel tubing packed with chromatographic substrate consisting of 10% dimethyl sulfolane on 42/60 mesh C-22 firebrick) bent into a "U" shape was fitted onto a gas sampling valve of the chromatograph.

For analysis, 100 ml gas samples were passed through the freeze trap which was immersed in a liquid O_2 bath. After concentration, the sample was volatilized at 0°C and injected into the chromatograph.

Concentrations of ethylene were estimated from the peak height of the traces on the gas chromatograph recorder charts, calibrated with known concentrations of ethylene. All measurements were made in Fort Collins, Colorado (.835 atmospheres) and were not corrected to standard temperature and pressure.

The treatment containers were opened periodically and the tubers examined for fungal growth, sprout growth, and general condition. Fungal growth was kept minimal by regular dustings with 10% Captan.

Results and Discussion

The effect of a flowing 2% O_2 atmosphere on endogenous ethylene production by potato tubers is illustrated in Fig. 2. Ethylene production at 32°F remained low and constant. No sprouting was observed at 32°F. At 45°F, ethylene production began to rise from about the eighth week and continued to increase until the experiment was terminated. The rise in ethylene production was associated with sprouting (Table 3).

The pattern of ethylene production in air was similar to that in 2% O_2 (Fig. 3). It must be noted, however, that the heavy dotted line is a postulated level of production. An ineffective ethylene scrubber masked the true values. This was later corrected for subsequent measurements. The measurements taken the 2nd week of storage are not in error. It is highly probable, therefore, that the level of ethylene production remained low and constant during the early weeks of storage (as was observed in 2% O_2, 4%CO_2, and intermittent air). Ethylene production at 32°F was low and steady throughout the seven month storage period. No sprouting occurred at 32°F but at 45°F sprouting occurred about the 14th week. The rise in ethylene production at 45°F was associated with sprouting.

Figure 4 shows the pattern of ethylene production by potato tubers stored in 80% O_2. Ethylene production at both 32°F and 45°F reached a peak about halfway through the study. No sprouting was

Table 3. Rate of sprout growth in inches of potato tubers in different storage atmospheres. Observations were made every three weeks for sprout and tuber condition.

Treat.	Temp.	Week of Storage						
		11	14	17	20	23	26	29
2% O_2	32° F	0	0	0	0	0	0	0
	45°	0	1/16	-	1-2	2-3	3-4	4
Air	32°	0	0	0	0	0	0	0
	45°	0	1/16	-	1-2	4-5	7-8	10+
80% O_2	32°	0	0	0	0	0	0	0
	45°	0	0	-	0	0	0	0
4% CO_2	32°	0	0	0	0	0	0	0
	45°	0	1/16	-	1	4-5	4-5	4-5*
12% CO_2	32°	0	0	0	0	0	0	0
	45°	0	0	-	0	0	0	0
Int. Air	32°	0	0	0	0	0	0	0
	45°	0	1/16	-	1	4-5	7-8	10+

* sprouts appeared necrotic at tips; some tubers completely devoid of any sprouts.

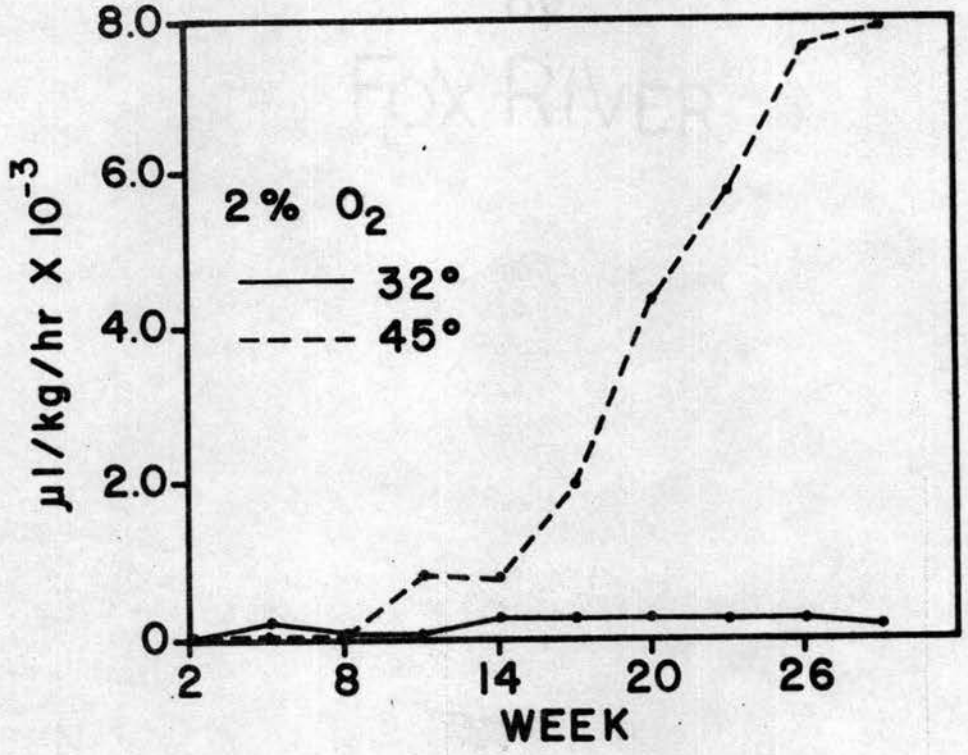


Figure 2. Changes in ethylene production by Russet Burbank tubers in 2% O_2 as influenced by storage time and temperature.

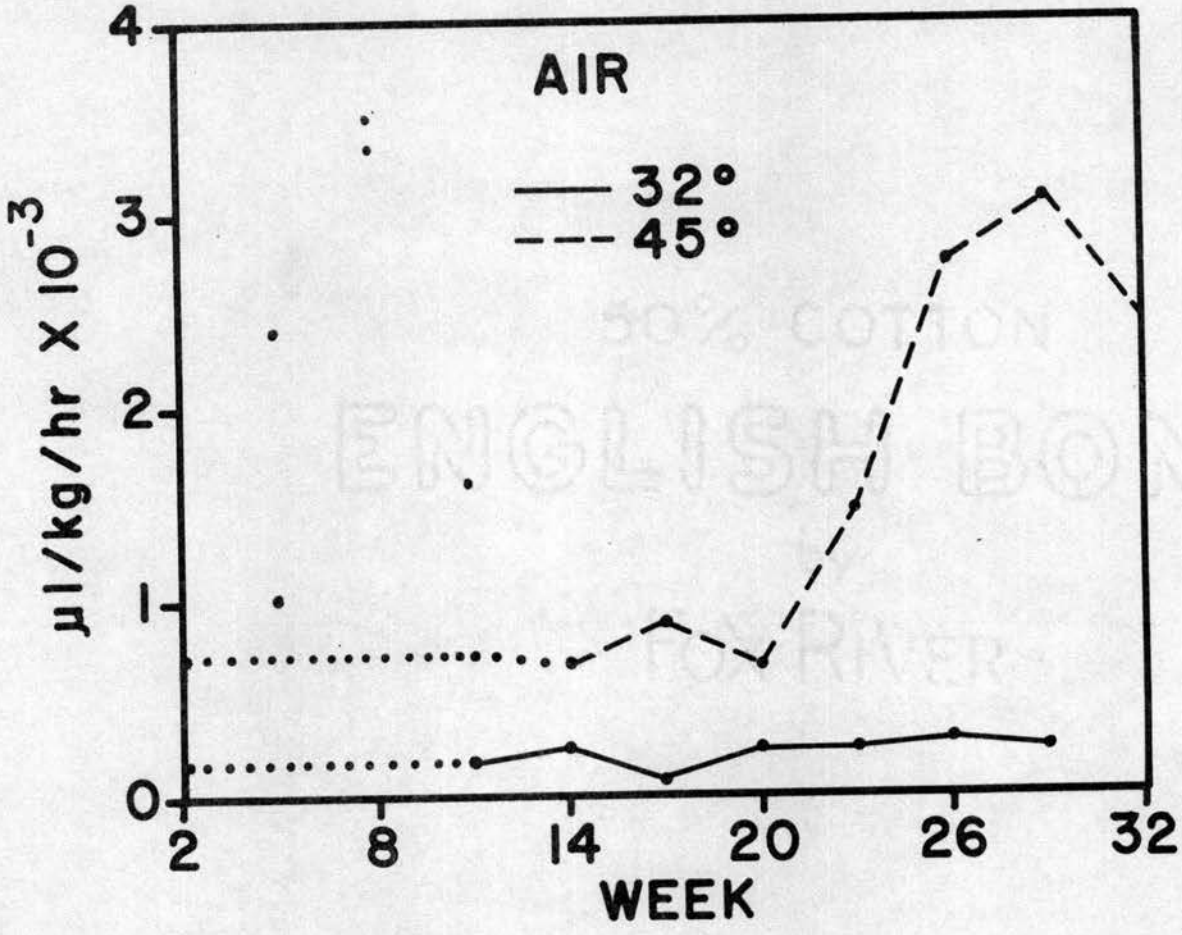


Figure 3. Changes in ethylene production by Russet Burbank tubers in flowing air as influenced by storage time and temperature. Dotted line indicates postulated level of ethylene production while the unconnected points are the levels measured.

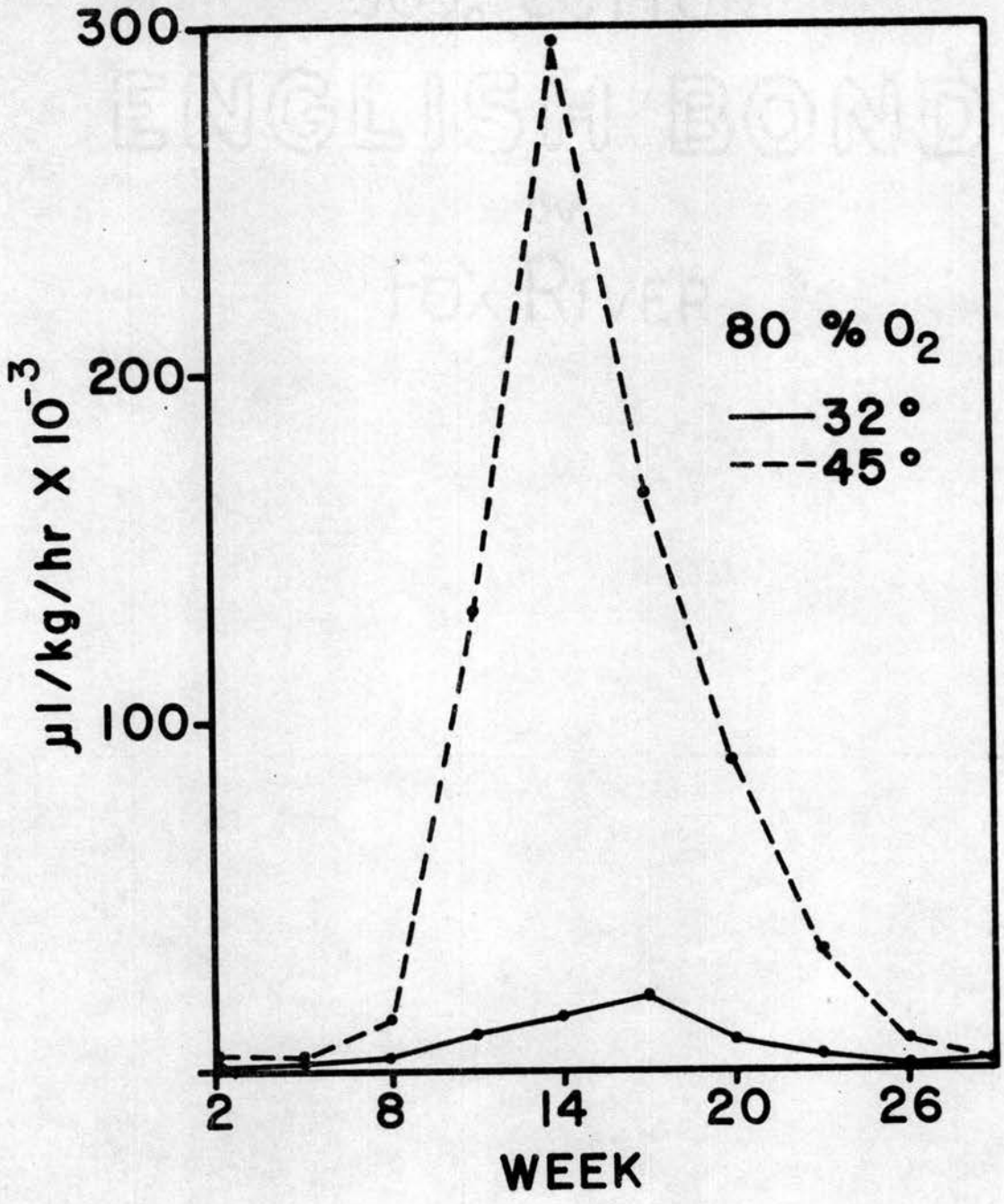


Figure 4. Changes in ethylene production by Russet Burbank tubers in 80% O_2 as influenced by storage time and temperature.

observed in either of the two temperatures. Tubers did not survive in 80% O_2 but instead underwent physiological and pathological breakdown. At 45°F ethylene production rose markedly from the eighth week until a peak of production was reached at about the 14th week of storage. It is important to note that the level of ethylene production at that point, 0.300 ul kg^{-1} hr^{-1}, is equivalent to that amount of production observed by McGlasson in his work on the wounding of potato tuber tissue (37). In that study, potato tubers were sliced into thin sections and the rate of ethylene production was traced. Therefore, potato tubers in 80% O_2 appear to be under a great deal of stress and respond to that stress by evolving ethylene at rates far above that of healthy tissue. The rate of ethylene production at 32°F, though not so dramatic as that observed in 80% O_2 at 45°F, was still much higher than the rate observed for tubers stored in 2% O_2, air, 4% CO_2, and intermittent air. At both temperatures the tubers succumbed to physiological breakdown and by the end of the study were in extremely poor condition. The decrease in the rate of ethylene production after the peak was reached at both temperatures may be due to the death of a greater and greater percentage of tissue until no tissue was physiologically active. Figure 5 illustrates the same treatments but with a different scale for ethylene production at 32°F.

The 4% CO_2 atmosphere was not toxic to potato tubers but did result in some inhibition of sprout growth. This atmosphere at 32°F

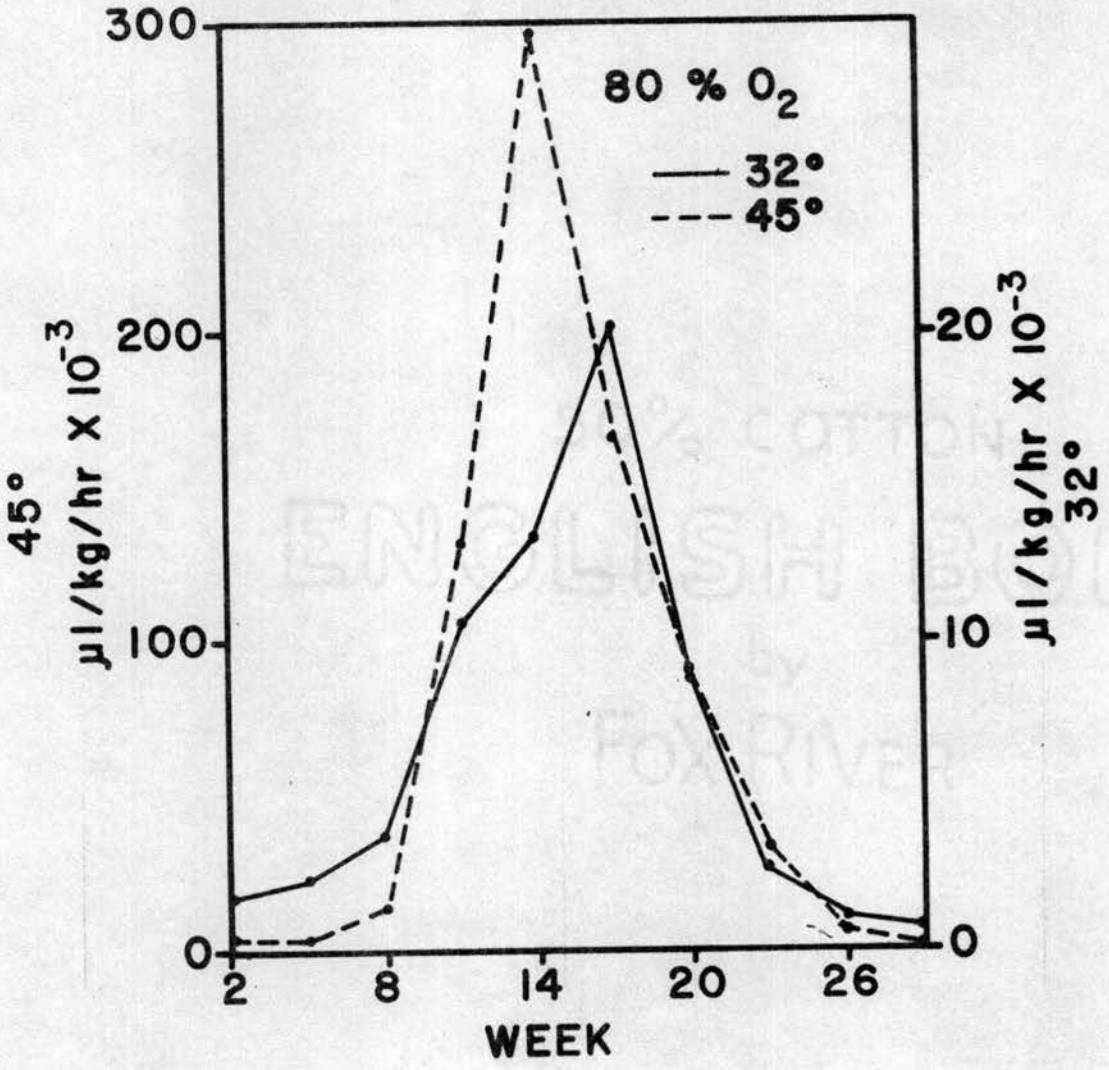


Figure 5. Changes in ethylene production by Russet Burbank tubers in 80% O_2 as influenced by storage time and temperature.

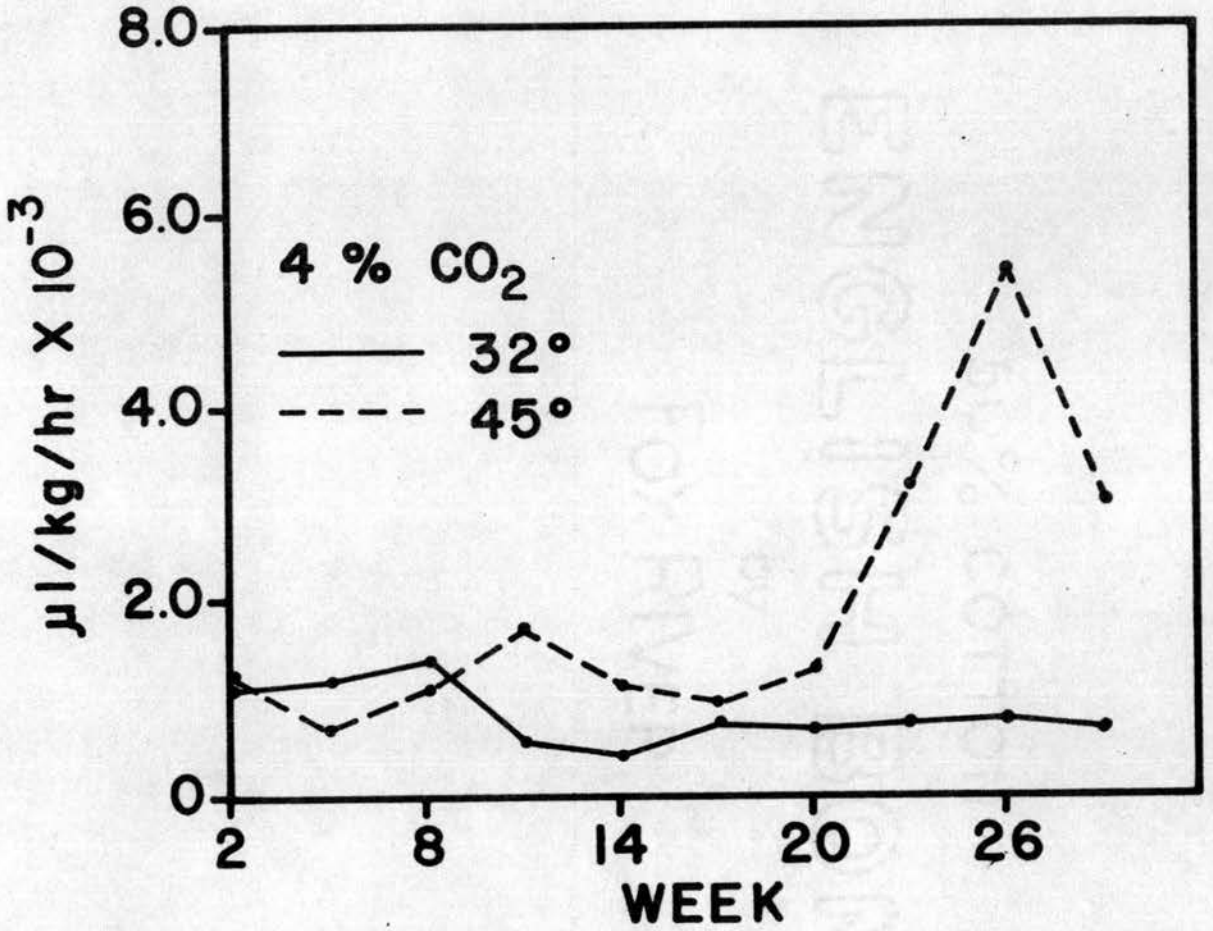


Figure 6. Changes in ethylene production by Russet Burbank tubers in 4% CO_2 as influenced by storage time and temperature.

induced a steady, low rate of ethylene production (Fig. 6). No sprouting was observed at 32°F. At 45°F ethylene production began to rise from about the 20th week and proceeded to increase until the 26th week. At that point a decrease occurred, possibly because sprout growth seemed to slow near the end of the experiment (Table 3).

Tubers stored in 12% CO_2 succumbed to this toxic atmosphere and underwent physiological and pathological breakdown. No sprouting occurred at either of the two temperatures. At 45°F ethylene production began slowly but rose sharply from the 20th week and continued to increase until the experiment was terminated (Fig. 7). The rise in ethylene production for tubers at 45°F was attributed to pathological invasion and its subsequent buildup since the tubers were in the advanced stages of physiological degeneration from the 17th week on. At 32°F the tubers evolved ethylene at a high rate initially (2nd week) and the rate of production increased until a peak of 0.025 ul kg^{-1} hr^{-1} was reached at the 14th week. It seems somewhat inconsistent that tubers in 12% CO_2 produced ethylene at a higher rate at 32°F than at 45°F. There are several explanations that lend themselves to this phenomenon. Potato tubers have a higher respiration rate at 32°F than at 41°F (71). What role this singular characteristic might play in the observed high rate of ethylene production is not known. Research has shown that the potato tuber can withstand a high CO_2 environment better at 45°F than at 32°F (70, 71).

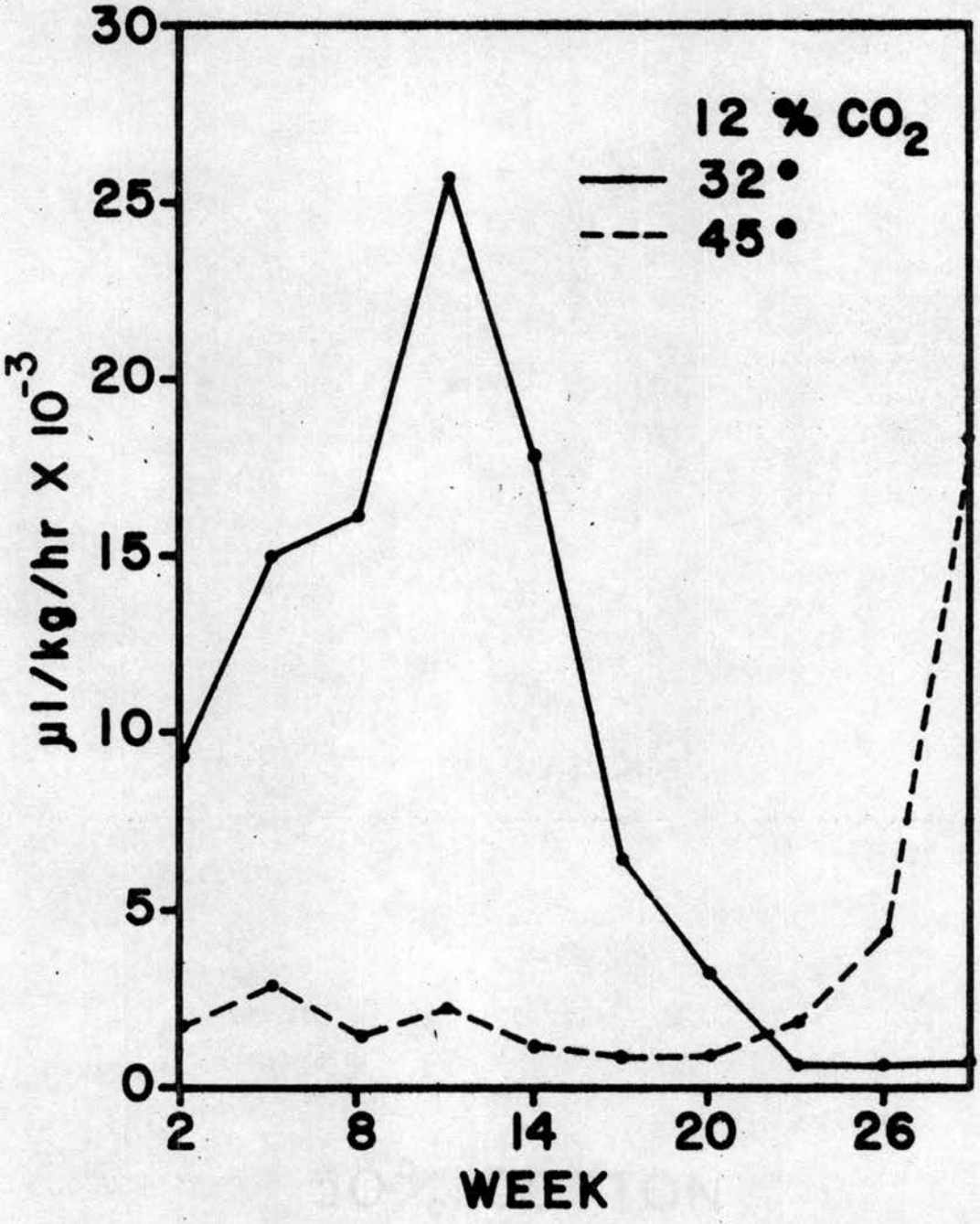


Figure 7. Changes in ethylene production by Russet Burbank tubers in 12% CO_2 as influenced by storage time and temperature.

The intermittent air treatment gave results similar to those observed for 2% O_2, flowing air, and 4% CO_2 (Fig. 8). The potato tubers at 32°F evolved ethylene at a low, steady rate. No sprouting was observed. At 45°F ethylene production began to rise from the 14th week to a peak of 0.007 ul kg^{-1} hr^{-1} at the 23rd week. Ethylene production then slowed until the experiment was terminated. The rise in ethylene production was associated with sprouting. No conclusions were reached for the observed decrease near the end of the experiment.

Figure 9 illustrates the great disparity between non-toxic atmospheres (2% O_2 and air) and toxic atmospheres (80% O_2) at 45°F. Two scales are used to illustrate the ethylene production changes for tubers in air and 2% O_2. The rates of ethylene production for tubers stored in 80% O_2 greatly exceeded the rates observed for tubers stored in air and 2% O_2.

Figure 10 reveals the effect of three oxygen treatments on endogenous ethylene production by tubers at 32°F. Tubers in air and 2% O_2 maintained steady, low rates of ethylene production throughout the entire seven month storage period. Tubers in 80% O_2 increased endogenous ethylene production until a peak was reached about the 17th week of storage.

Of the three CO_2 treatments at 45°F no real differences can be seen (Fig. 11). Although the 12% CO_2 atmosphere was toxic to the tubers, ethylene production remained very low and steady until physiological and pathological breakdown occurred.

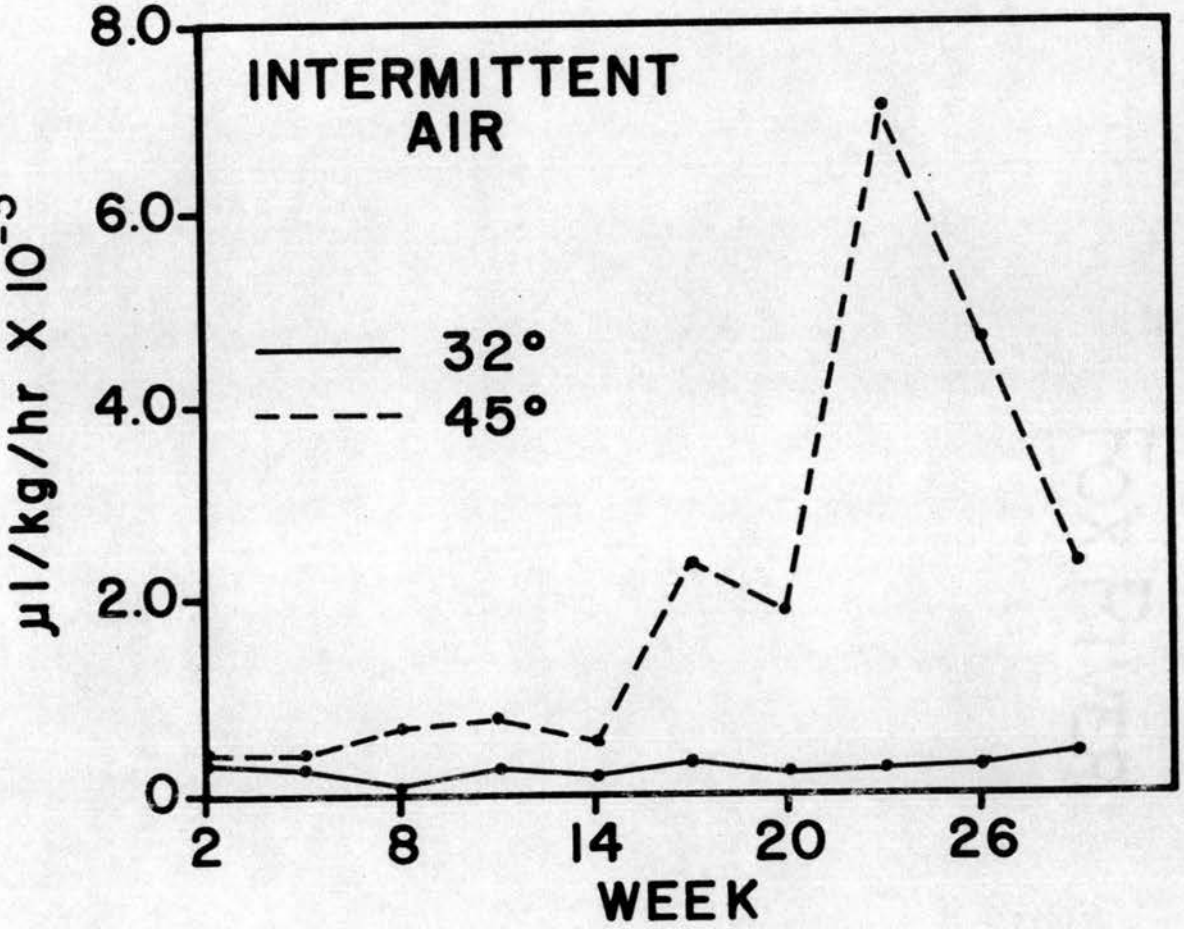


Figure 8. Changes in ethylene production by Russet Burbank tubers under intermittent air conditions (containers sealed 48 hours, flushed with air, sealed 48 hours, flushed, etc.) as influenced by storage time and temperature.

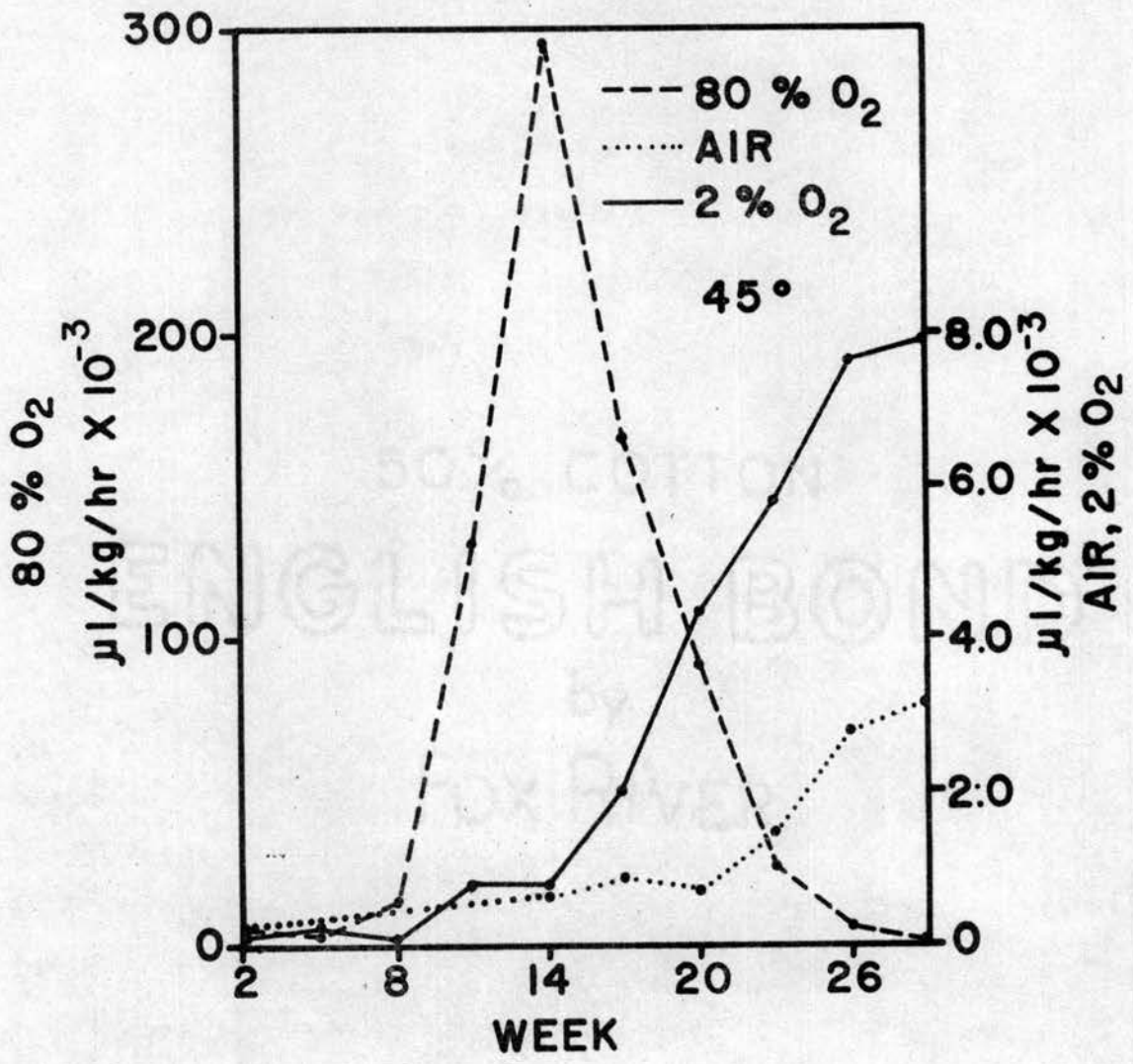


Figure 9. Changes in ethylene production by Russet Burbank tubers at 45°F in 80% O_2, flowing air, and 2% O_2 as influenced by storage time. Heavy dotted line on air curve is postulated level of ethylene production.

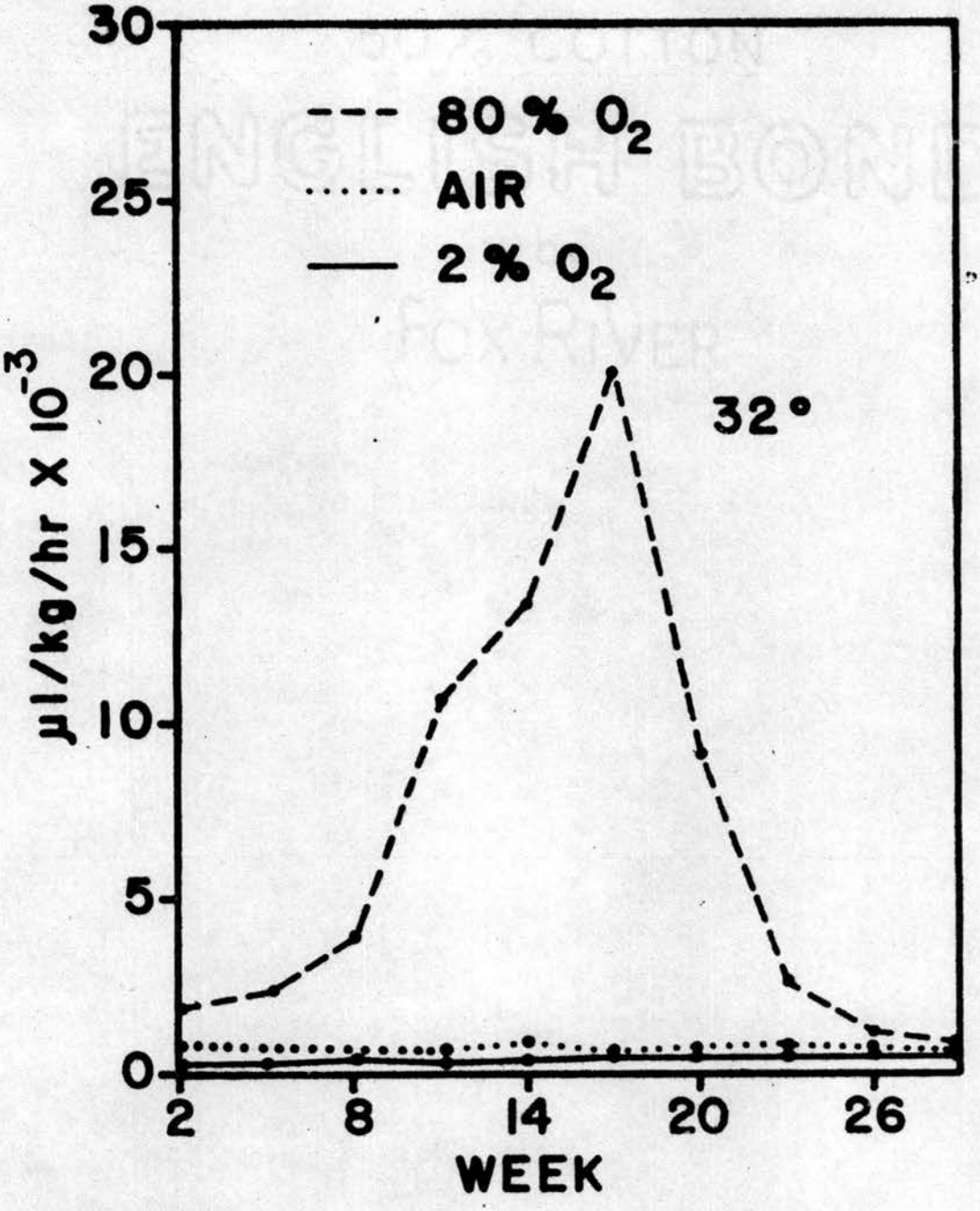


Figure 10. Changes in ethylene production by Russet Burbank tubers at 32°F in 80% O_2, flowing air and 2% O_2 as influenced by storage time. Heavy dotted line on air curve is postulated level of ethylene production.

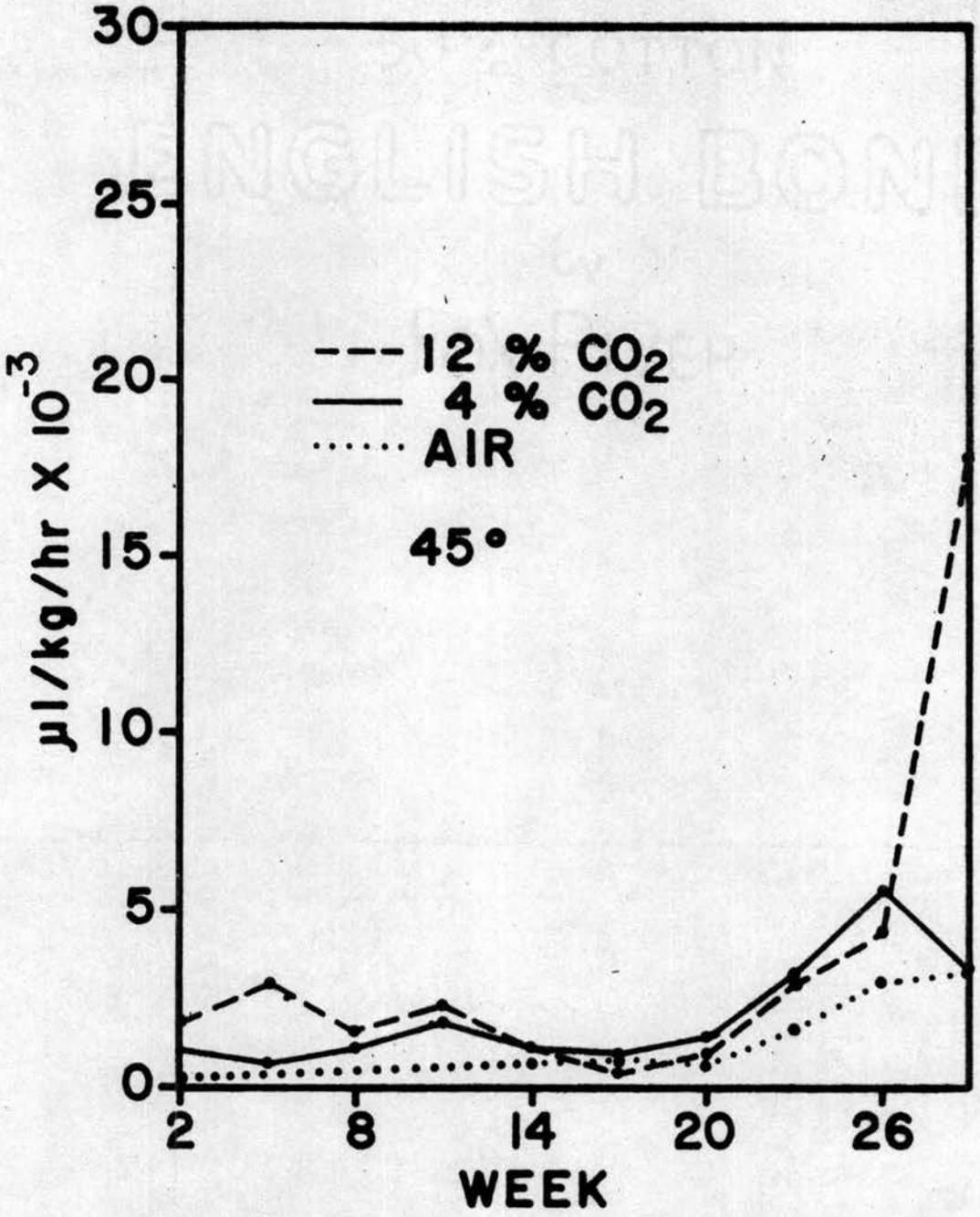


Figure 11. Changes in ethylene production by Russet Burbank tubers at 45°F in 12% CO_2, 4% CO_2, and flowing air as influenced by storage time. Heavy dotted line on air curve is postulated level of ethylene production.

The three CO_2 treatments at 32°F revealed the difference between a toxic atmosphere (12% CO_2) and non-toxic atmospheres (air and 4% CO_2). Figure 12 illustrates that 12% CO_2 was the only concentration of CO_2 capable of initiating high endogenous rates of ethylene production at this temperature. Tubers stored in air and 4% CO_2 maintained steady, low rates of ethylene production.

Summary and Conclusions

Potato tubers stored at temperatures and atmospheres most likely encountered in commercial storage bins evolved ethylene at rates usually less than 0.005 ul kg^{-1} hr^{-1}. Endogenous ethylene production rose slightly in those treatments in which sprouting occurred. Potato tubers stored in the toxic atmospheres, 12% CO_2 and 80% O_2, evolved ethylene at rates far above that of tubers stored in non-toxic atmospheres. Tubers in 80% O_2 evolved ethylene at a much higher rate at 45°F than at 32°F. Tubers stored in 12% CO_2, however, evolved ethylene at a higher rate at 32°F than at 45°F.

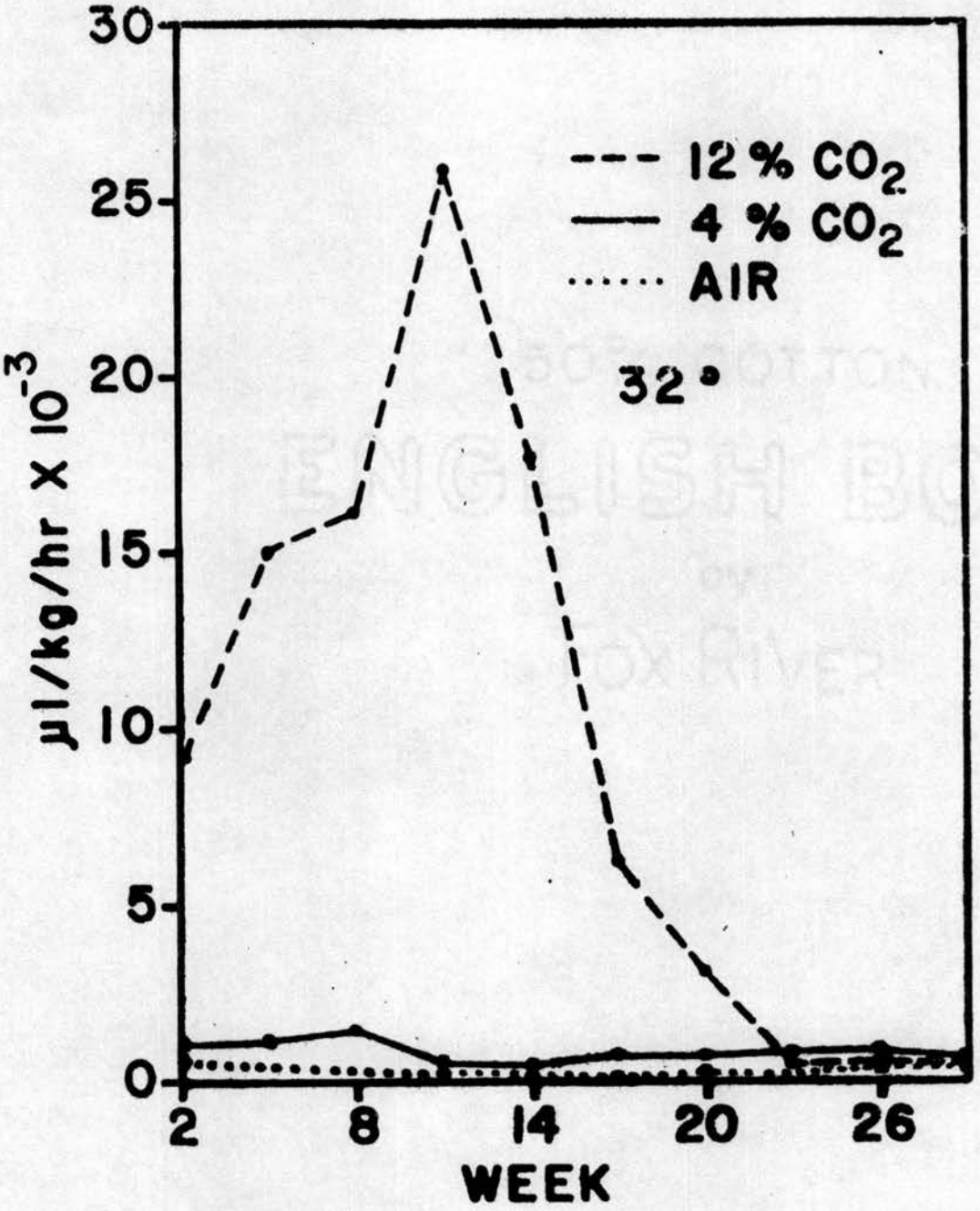


Figure 12. Changes in ethylene production by Russet Burbank tubers at 32°F in 12% CO_2, 4% CO_2, and flowing air as influenced by storage time. Heavy dotted line on air curve is postulated level of ethylene production.

IV. THE EFFECT OF TWO PATHOGENS ON ETHYLENE PRODUCTION BY POTATO TUBERS

Objective

The purpose of this portion of the study was to gain an understanding of the influence of two common storage pathogens on inducing ethylene production by potato tubers.

Experimental

Russet Burbank tubers grown in the San Luis Valley, Colorado in 1970 and stored for seven months at 45°F were selected for Part A of the study. Norchip potatoes grown in 1971 in Weld county, Colorado were selected for Part B of the study and were used immediately after harvesting.

Part A. Approximately 1 kg of potato tubers were placed in 4 liter jars and sealed with a metal lid equipped with inlet and outlet ports. The jars containing the tubers were then flushed with ethylene-free air at a flow rate of 24 liters/hour for 36 hours to remove the ethylene from the internal tissue of the tubers. The tubers were then removed from the jars and inoculated with two common storage pathogens, Fusarium roseum var. sambucinum (LK) Sn. and H. and Alternaria solani (Ell. & G. Martin) L. R. Jones and Grout. Inoculation was

accomplished with a cork borer (1/8th inch OD) in three areas of the tuber. Holes, one-quarter inch deep, were cut in the apical end of the tuber, in the terminal end, and halfway between the two. Using the cork borer and aseptic techniques, 1/8th inch diameter plugs of inoculum were taken from cultures of each pathogen grown on PDA in petri dishes. Single plugs of inoculum were placed in each of the three holes on each tuber. The tubers were then returned to each jar and a beaker of KOH pellets was added to remove CO_2. The jars were flushed with ethylene-free air for several minutes to remove any background levels of ethylene. The jars were sealed and a rubber septum was fitted onto one port to permit the collection of gas samples. A manometer was fitted on the remaining port (Fig. 13). The manometer indicated the rate of oxygen consumption by the tubers (and, therefore, the amount of oxygen needed to maintain atmospheric levels in the jars). Two jars were used per treatment with eight tubers per jar (Table 4).

Part B. Freshly harvested Norchip potatoes were used for this study. The manner of preparation was essentially the same as Part A. However, the Alternaria solani treatment was replaced with a treatment to assess the ethylene-producing ability of PDA-grown Fusarium roseum var. sambucinum. One petri dish containing the culture was placed into each of two jars; the Fusarium roseum culture completely occupied the 61 cm^2 of the petri dish.

Figure 13. Schematic design of experiment: A) 4 liter jar equipped with inlet and outlet ports, a manometer, and a flask of KOH to remove CO_2 B) petri dish with culture and cork borer by which inoculations were made C) syringe for the collection of gas samples.


A
B
C

Table 4. Treatments included in pathological study. Russet Burbank tubers were used in Part A and Norchip tubers in Part B. Treatments kept at 55°F.

Treatment	Rep	Tubers inoculated with	Tuber wt in kg
Part A			
1	1	0	1.055
	2	0	1.035
2	1	*Alternaria solani*	1.065
	2	" "	1.050
3	1	*F. roseum* var. *sambucinum*	0.995
	2	"	1.080
Part B			
4	1	0	1.042
	2	0	1.044
5	1	*F. roseum* var. *sambucinum*	1.022
	2	"	1.020
6	1	*F. roseum* on PDA medium	-*
	2	"	-*

* treatment 6 used 61 cm^2 of PDA-grown *Fusarium roseum* var. *sambucinum* per rep in petri dishes. Treatments 2, 3, and 5 received a total inoculation of less than 2 cm^2 per rep.

In Part A and Part B ethylene measurements were made every 24 hours for 12 or 13 days in the manner described in Section III. Oxygen consumption by the tubers in each jar was indicated by the manometer and replaced by injections of ethylene-free O_2. Ethylene produced by the tubers and/or pathogens accumulated in the 4 liter enclosure. Fifty ml gas samples drawn from the treatment jars were analyzed for ethylene. Fifty ml of ethylene-free air were then added to each jar to replace that removed.

Results and Discussion

Table 5 indicates the daily ethylene evolution rates for the various treatments of Part A (on a ul kg^{-1} day^{-1} basis). Tubers inoculated with Fusarium roseum var. sambucinum began to produce ethylene at higher than control rates from the third day. The uninoculated tubers maintained a steady rate of ethylene production (average rate for 12 days: 0.060 ul kg^{-1} day^{-1}). Tubers inoculated with Alternaria solani responded very much the same as the uninoculated tubers (average rate for 12 days: 0.091 ul kg^{-1} day^{-1}). Tubers inoculated with F. roseum evolved ethylene at very high rates (highest observed rate: 1.080 ul kg^{-1} day^{-1}). Figure 14 illustrates the accumulation of ethylene in the 4 liter containers.

Part A indicated that Fusarium roseum var. sambucinum greatly stimulated ethylene production when inoculated onto Russet

Table 5. The daily rate of ethylene evolution by uninoculated Russet Burbank tubers, by tubers inoculated with Alternaria solani, and by tubers inoculated with Fusarium roseum. Each value is average of two samples. Treatments were held at 55°F. Underlined readings are the peak ethylene production rates observed.

	Treatment		
	Ethylene production rate (ul kg^{-1} day^{-1})		
Days at 55°F	Control*	Alt. solani	F. roseum
1	.048	.044	.074
2	.041	.033	.031
3	.034	.041	.150
4	.059	.041	.539
5	.055	.070	.627
6	.052	.065	<u>1,084</u>
7	<u>.122</u>	.164	.651
8	.088	<u>.177</u>	.624
9	.015	.096	.278
10	.055	.063	.403
11	.082	.166	.367
12	.055	.148	.305

* the average ethylene production rate for 12 days: 0.060 ul kg^{-1} day^{-1}.

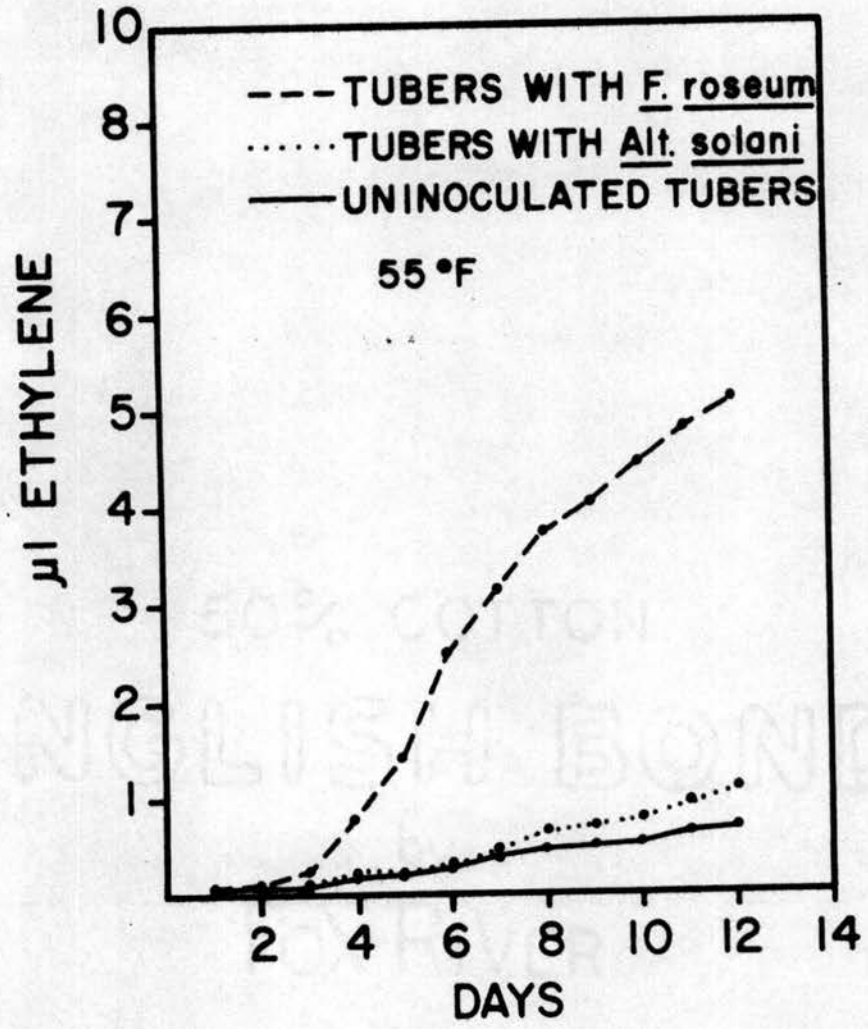


Figure 14. Accumulation in sealed 4 liter jars of ethylene produced by uninoculated Russet Burbank tubers, by tubers inoculated with Alternaria solani, and by tubers inoculated with Fusarium roseum var. sambucinum at 55°F as influenced by time.

Burbank tubers. With this knowledge, Part B was designed to repeat much of Part A. Norchip potatoes were used in Part B and the results when compared to Part A indicated higher levels of ethylene production for both inoculated and uninoculated tubers (Fig. 15). It did not seem that there was a qualitative difference in the rate of decay between the two varieties. The differences in variety alone might account for the disparity of ethylene production rates; Russet Burbank tubers were used in Part A and Norchip tubers in Part B. The physiological and chronological ages of the two varieties of tubers used were different; the Russet Burbanks had undergone seven months storage at 45°F while the Norchips were used immediately after harvesting. Although the rates of ethylene production were different in the two studies, the trends remained the same. Table 6 indicates the daily ethylene evolution rates for the various treatments. Figure 15 illustrates the accumulation of ethylene in the 4 liter jars. Again, the tubers inoculated with F. roseum var. sambucinum began a dramatic increase in ethylene production from the third day that continued until the experiment was terminated. The uninoculated tubers maintained a fairly steady rate of ethylene production (average rate for 13 days: 0.216 ul kg^{-1} day^{-1}).

The most interesting aspect of Part B was the almost non-existent production of ethylene by F. roseum in culture. This was true even though the amount of mycelium in this treatment was over

Table 6. The daily rate of ethylene evolution by uninoculated Norchip tubers, by tubers inoculated with Fusarium roseum var. sambucinum, and by 61 cm^2 of PDA-grown F. roseum var. sambucinum culture. Each value is average of two samples. Treatments held at 55°F. Underlined readings are peak ethylene production rates observed.

	Treatments		
	Ethylene production rate (ul kg^{-1} day^{-1})		
Days at 55°F	Control*	Tubers with F. roseum	PDA-grown F. roseum**
1	.170	.148	.012***
2	.089	.090	.027
3	.054	.142	-.007
4	.112	.581	.004
5	.109	.349	.007
6	.102	.977	.002
7	.202	.919	.006
8	.141	.440	.002
9	.347	1.346	.005
10	.189	1.377	.008
11	.437	3.303	.000
12	.439	2.742	.003
13	.302	1.341	.006

* the average ethylene production rate for 13 days: 0.216 ul kg^{-1} day^{-1}.

** 61 cm^2 of PDA-grown F. roseum var. sambucinum culture.

*** first three readings were unstable; from the fourth day on readings stabilized. Since levels of ethylene were so low, diffusion from the ambient air may have occurred.

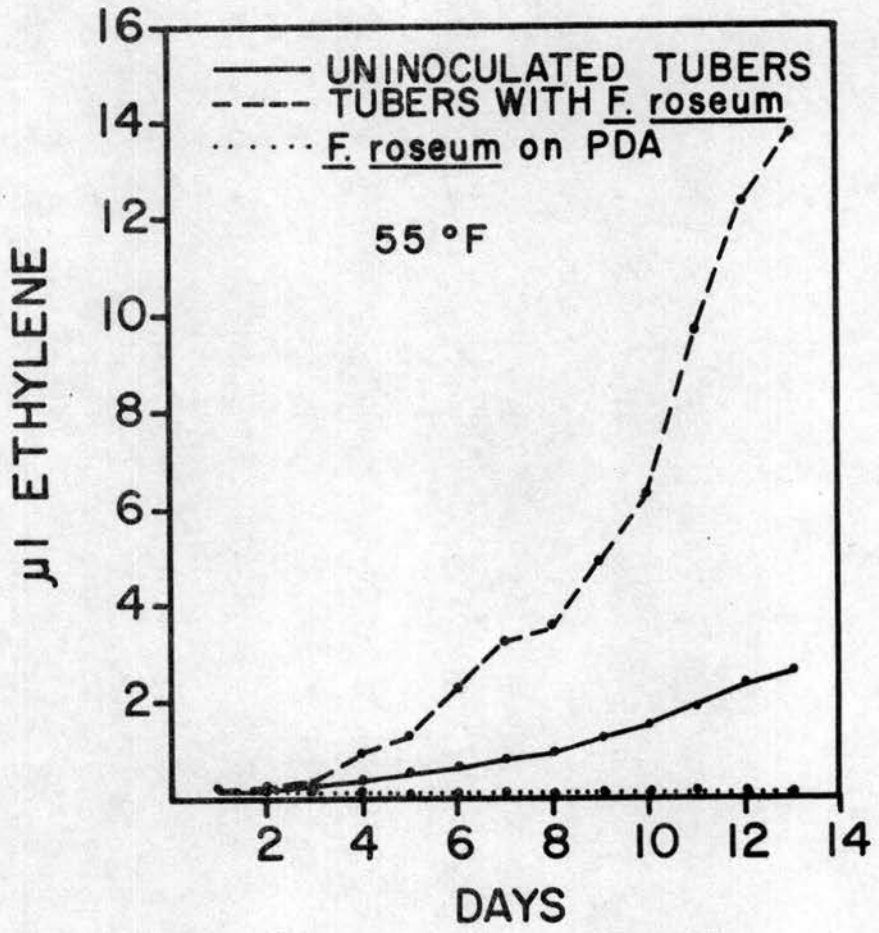


Figure 15. Accumulation in sealed 4 liter jars of ethylene produced by uninoculated Norchip tubers, by tubers inoculated with Fusarium roseum var. sambucinum, and by PDA-grown F. roseum cultures at 55°F as influenced by time.

25 times that of the initial amount used to inoculate tubers in treatment 5 (61 cm^2 vs. 2 cm^2). Any explanation for this must be cautious: the PDA did not contain any of the accepted precursors of ethylene (methionine, linolenic acid, various Kreb's cycle acids). The cultures when placed into the jars were no longer spreading, having just filled the confines of the petri dish. Ethylene production may require actively growing Fusarium roseum (as the case would be on inoculated tubers).

Summary and Conclusions

Part A and Part B of the pathological study indicated consistently that tubers inoculated with Fusarium roseum var. sambucinum evolved ethylene at rates much greater than uninoculated tubers. Tubers inoculated with Alternaria solani evolved ethylene at a rate similar to uninoculated tubers. Cultures of F. roseum grown on PDA failed to produce ethylene.

V. DISCUSSION

The most significant observations made in this work were:

1) Russet Burbank tubers under conditions most likely encountered in commercial storages evolved ethylene at very low rates, usually below 0.005 ul kg^{-1} hr^{-1} (Figures 3, 6, 8).

2) Potato tubers stored in toxic atmospheres demonstrated the capacity to evolve ethylene at very high rates (Figures 5, 7).

3) In all cases where sprouting occurred, the ethylene production rate increased slightly (Figures 2, 3, 6, 8).

4) The dry rot organism, Fusarium roseum var. sambucinum, inoculated onto two varieties of potatoes greatly stimulated ethylene production by the tubers (Figures 14, 15).

These observations coupled with the results of other researchers studying ethylene and the potato tuber provide a basis of understanding from which conclusions can now be reached.

McGlasson in 1969 (37) attributed an ethylene production rate of less than 0.015 ul kg^{-1} hr^{-1} to intact, dormant tubers (Solanum tuberosum L., cv. Bungama). In this work, tubers stored in 2% O_2, flowing air, 4% CO_2, and in sealed containers evolved ethylene at rates less than 0.005 ul kg^{-1} hr^{-1}. It is possible that different varieties of potato tubers evolve ethylene at different rates. Norchip

potatoes in sealed jars produced ethylene at an average rate of 0.009 ul kg^{-1} hr^{-1}.

The rise in ethylene production upon sprouting is a phenomenon that merits discussion. Poapst in 1968 (45) observed that tubers treated with gibberellic acid developed higher internal levels of ethylene than untreated tubers. Bruinsma in 1962 (6) related that the gibberellin content of stored tubers increased more than thirty fold upon sprouting. The observed change in ethylene production upon sprouting in this work was real but not dramatic. The role of trace amounts of evolving ethylene is unknown. It is possible that prior to sprouting gibberellins initiate higher ethylene production rates which then initiate sprouting. The sequence of gibberellin-ethylene-sprouting is only speculative and merits further study.

Low oxygen concentrations have been related to lowered ethylene production rates for many tissues. Burg and Thimann in 1959 (7) observed a 50% reduction in ethylene production and respiration for apple tissue slices placed in a 2.5% O_2 atmosphere. Mapson (38) reported that bananas stored in low oxygen environments refused to ripen; ethylene production was drastically reduced. In this work, potato tubers stored in 2% O_2 evolved ethylene at extremely low rates at 32°F and 45°F (less than 0.001 ul kg^{-1} hr^{-1} through 14 weeks of storage). It is interesting to note that upon sprouting a rise in the rate of ethylene production occurred despite the low level of oxygen

present. Comparisons of ethylene production rates for tubers stored in 2% O_2, air, 4% CO_2, and intermittent air revealed the same trend. The ethylene evolution rate for tubers stored in 2% O_2, although minuscule, was not dramatically lower than the rates at other atmospheres. All treatments evolved ethylene at rates less than 0.002 ul kg^{-1} hr^{-1} through the first 14 weeks of storage (excepting, of course, the two toxic treatments, 80% O_2 and 12% CO_2). The differences that did occur at those low rates were not analyzed for significance due to the profile of the experiments.

The dramatic levels of ethylene production observed for potato tubers stored in 80% O_2 at 45°F demonstrated the presence of a mechanism in potato tubers for the production of ethylene. Biale, Young, and Olmstead in 1954 (4) found that oranges placed into 100% O_2 evolved ethylene at much higher than normal rates. It was hypothesized that higher-than-atmospheric concentrations of oxygen initiated increased rates of ethylene production which in turn caused an acceleration of respiration. In this work, tubers stored in 80% O_2 at 32°F evolved ethylene at high rates but not nearly so dramatic as the rates observed at 45°F (up to 0.300 ul kg^{-1} hr^{-1}). This suggests the influential nature of temperature.

Tubers stored in 12% CO_2 produced ethylene at higher rates at the lower temperature, 32°F, than at the higher temperature, 45°F. Freezing or chilling injury is not a probable cause for this anomaly. The potato tuber's unique characteristic of respiring more at 32°F

than at 41°F perhaps played a role in this phenomenon. Workman demonstrated that potato tubers stored in high CO_2 environments held up better at 41°F than at 32°F (71). The reasons for this are not altogether understood.

The system described for the analysis of ethylene production by different pathogens proved to be efficient and relatively foolproof. The dry rot organism, Fusarium roseum var sambucinum, greatly stimulated ethylene production when inoculated onto potato tubers. Potato tubers inoculated with Alternaria solani evolved ethylene at rates not dissimilar to uninoculated tubers.

In no case did unstressed tubers evolve ethylene at rates higher than 0.010 ul kg^{-1} hr^{-1}. Sprouting tubers closely approached this level but did not exceed it. Tubers stored in 80% O_2 at 45°F evolved ethylene at a peak rate of 0.300 ul kg^{-1} hr^{-1}; at 32°F the peak rate was 0.020 ul kg^{-1} hr^{-1}. In 12% CO_2 at 32°F tubers produced ethylene at a peak rate of 0.026 ul kg^{-1} hr^{-1}. The maximum rate of ethylene production observed for tubers inoculated with Fusarium roseum exceeded 0.100 ul kg^{-1} hr^{-1}. A common basis for high endogenous rates of ethylene production by potato tubers would require stress conditions on the tubers.

It is interesting to note that even low rates of ethylene production could result in accumulations that are physiologically significant. A typical storage bin with dimensions 60' x 30' x 20' may hold 1500

sacks or 150,000 pounds of potatoes. If an ethylene production rate of 0.005 ul kg^{-1} hr^{-1} is assumed for the tubers, the concentration in the bin would build to about 100 ppb in 24 hours (assuming no ventilation and no diffusion). If the tubers were infected with Fusarium roseum and evolved ethylene at a rate of 0.100 ul kg^{-1} hr^{-1} (highest observed rate for infected Norchips: 0.130 ul kg^{-1} hr^{-1}), then the concentration in the bin would exceed 100 ppb in only 2 hours. It is possible, therefore, that endogenous ethylene production by potato tubers could result in physiologically significant concentrations.

VI. SUMMARY AND CONCLUSIONS

1. Russet Burbank tubers stored in atmospheres of 2% O_2, air, 4% CO_2, and intermittent air at 45°F and 32°F evolved ethylene at a rate no greater than 0.008 ul kg^{-1} hr^{-1} throughout a seven month storage period.
2. In all cases where sprouting occurred, the rate of ethylene production was observed to increase.
3. Russet Burbank tubers stored in 80% O_2 at 45°F and 32°F evolved ethylene at rates much higher than the rates observed for tubers stored in 2% O_2, air, 4% CO_2, and intermittent air.
4. In general, the higher temperature produced the higher rates of ethylene production. The only exception to this rule were those tubers stored in 12% CO_2.
5. Tubers inoculated with Alternaria solani evolved ethylene at a rate similar to that of uninoculated tubers. Tubers inoculated with Fusarium roseum var. sambucinum evolved ethylene at a rate far greater than that observed for uninoculated tubers.

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