PATHOLOGICAL RESPIRATION AND SYSTEMIC FACTORS IN FUSARIUM WILT OF TOMATO

By

RALPH PORTER COLLINS

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
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RALPH PORTER COLLINS ABSTRACT

Leaves from infected plants had higher respiration than disease-free controls as early as 1 day after inoculation with Fusarium oxysporum f. lycopersici. Higher respiration rates continued until plants showed advanced symptoms. These comparative results were obtained using leaf discs with Warburg technique. The relationship held whether respiration was determined on a dry weight or on a nitrogen basis. Total nitrogen was unchanged 2 and 8 days after inoculation but on the 11th and 15th days leaf tissue from diseased plants showed a 10 to 18 per cent increase in total nitrogen. Since leaves were not invaded by the fungus the findings indicate systemic toxemia.

The respiration of stem slices followed a similar pattern. The pathogen was present in stems in the area used as early as 2 days after inoculation, but all stem sections did not contain the pathogen until 7 days after inoculation.

Several substances known to be produced by F. oxysporum f. lycopersici were tested for their effect on host respiration. Fusarinic acid at concentrations of 10^{-2} M, 10^{-3} M, and 10^{-4} M had an inhibitory effect on leaf respiration. In lower concentration fusarinic acid had no effect. Pectinase, a commercial enzyme preparation consisting of several pectolytic enzymes, had no effect on respiration of leaf discs taken from cuttings regardless of the concentration used. Cuttings were allowed to take up ethylene solutions by tran-

spirational pull and respiration of leaves was determined. Leaf discs were infiltrated with ethylene solutions, and leaves were also gassed with ethylene. Respiration was unaffected regardless of the treatment or the concentration of ethylene used.

Infection caused increased permeability to electrolytes as early as 4 days after inoculation as determined by conductivity of leaf leachings. Permeability thus determined was approximately double that of the controls 12 days after inoculation.

An attempt was made to identify ethylene as a volatile from diseased plants using gas chromatography and infrared spectroscopy. Results were inconclusive. A bacterial contaminant growing on tomato leaf tissue produced a volatile identified as nitrous oxide.

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INTRODUCTION

It has been thought that wilt inducing pathogens produce toxins which are liberated into the host and cause symptoms in advance of the fungus (20). Most experimental work on toxins is based on the assumption that symptoms of disease are specific and that metabolites of the fungus produced *in vitro* are formed in diseased plants and have a causal relationship with disease. There are no data to support these assumptions. In the present work the role of toxins in tomato plants systemically invaded by *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder and Hansen, has been evaluated using host respiration and permeability as criteria. The role of fusarinic acid and ethylene, products of the fungus on the host, was studied in these respects. During the study, still other effects of ethylene became apparent and were evaluated.

Metabolic changes in plants infected by obligate parasites (2) and in fruit and storage organs invaded by facultative saprophytes (49) have been studied. Other types of diseases have had little attention. Possible important similarities and differences between infection by obligate parasites and vascular fusaria were studied.

LITERATURE REVIEW

Respiratory changes in diseased plants.

The respiration of plants infected with obligate parasites has been studied extensively and several reviews are available (4, 5, 42). In general, respiratory increases have been noted in plants infected by strict parasites. However, in Prasium majus (L.) infected with Erysiphe lamprocarpa (Kickx.), in Torilis nodosa (Gaertner) infected with Erysiphe communis (Wallr.)Fr.) (34), and in bean leaflets infected with Erysiphe polygoni (DC) (35) decreased respiration was reported.

Respiratory increases in wheat leaves infected with powdery mildew were shown by Allen (2) and by Yarwood (55) to occur in uninvaded cells of the host. From these experiments Allen et al. concluded that toxic substances produced by the mildew diffuse into underlying host cells and cause changes in both anaerobic and aerobic respiration. Mildewed leaves of clover dusted with sulphur to kill the mildew maintained an increased respiration. This indicated that increased respiration of mildewed leaflets was largely due to the stimulation of host respiration by the mildew rather than by respiration of the mildew fungus (55). Areas of diseased leaves removed from the affected zone failed to show an increase in respiratory rate indicating that the spread of the mildew toxins was restricted (2, 3). Experiments utilizing tagged substrates indicated that a diffusible substance (or substances) produced at the cite of incipient infection by

the host cells, by the fungus, or by both enhanced the metabolic activity of the host tissue in the area of infection. The accumulation of various substances by leaves of wheat and barley infected with Puccinia and Erysiphe was dependent upon aerobic respiration (43).

The nature of the increased respiration in plants infected with obligate parasites is not clearly understood but several theories have been proposed. It is said that increased respiration of infected wheat leaves is due to an inhibition of the Pasteur effect. The Pasteur effect is the suppression of carbohydrate breakdown aerobically as compared with the rate of breakdown anaerobically. In wheat infected with powdery mildew an increase in inorganic phosphate and an inhibition of the Pasteur effect was found (38). Allen held that mildew toxins acted as uncopulers and accelerated the release of inorganic phosphate and regeneration of ADP. He also interpreted Sempio's data (42) as indicating an inhibition of the Pasteur effect.

Other workers have followed Allen's ideas on inhibition of the Pasteur effect. Farkas and Kiraly (17) found that wheat infected with stem rust and powdery mildew became progressively less sensitive to malonate. At the same time glycolysis inhibitors suppressed oxygen uptake equally in healthy and diseased plants. This is interpreted to be a completion of glycolysis and oxidation of glycolytic products without participation of the tricarboxylic acid cycle (17). These ideas appear consistent with the idea of Pasteur effect

inhibition since Pasteur effect means inhibition of fermentation under aerobic conditions.

The increase in respiration in rust-infected plants is thought by Daly et al. (11) and by Shaw and Samborski (43) to occur via the "hexose monophosphate shunt". It was observed that per unit of oxygen consumed the number 1 carbon atom of glucose supplied exogenously is oxidized to carbon dioxide faster by rusted and mildewed leaves than by healthy leaves (43). It was also shown that the relative contribution of the number 1 carbon atom of glucose to the amount of carbon dioxide evolved increased with the incidence of disease. However, the hexose monophosphate shunt may be operative in the pathogen rather than in the invaded tissue, since Shu et al. have shown that this pathway is followed by germinating rust spores (44). In uninfected tissue the Embden-Meyerhof pathway was predominant.

The respiratory pattern of plants infected with facultative saprophytes is similar to that of obligate parasites. Carbon dioxide production by half roots of sweet potatoes infected with Rhizopus tritici (Saito) was higher than the controls 1 day after inoculation and by the second day was 9 times that of the uninfected half (50). Potato tissues infected with Bacillus phytophthorus (Appel.) also showed large increases in respiration (15). Comparison of respiratory rates of tobacco tissue infected with Phytophthora parasitica var. nicotianae (Tucker) and healthy tissue showed increased respiratory rates of invaded tissues (53). Since

it was impossible to separate host respiration from the pathogen respiration some of the increase was undoubtedly due to the fungus. Sweet potatoes infected with Ceratostomella fimbriata (Elliott) had a greatly augmented respiration (6, 7, 48).

The role of toxin production in a disease caused by a facultative saprophyte was studied by Uritani and colleagues (6, 7, 8, 48). Several abnormal metabolites were found in sweet potatoes infected with Ceratostomella fimbriata. One of them, ipomeamarone, a sesquiterpene, accelerated the respiration of sweet potato slices by 30-40 per cent in a solution of 1:4,000 dilution (48). Ipomeamarone stimulated not only the respiration of sweet potato slices but acted as an uncoupler of oxidative phosphorlyation carried out by cytoplasmic particles of mung bean and sweet potato. It was suggested that respiratory changes in Ceratostomella infected sweet potatoes is due to inhibition of the Pasteur effect thus following the pattern found in rust and powdery mildew infected plants. In later work, the Japanese workers found that ipomeamarone action probably accounted for only a small percentage of the respiratory increase. Tissue adjacent to the area of infection had a significant increase in functional protein and an increased activity per unit of protein (7, 8).

The respiratory changes in Fusarium wilt of tomato, a systemic invader, have received little attention. Bloom (10) measured the rate of respiration of leaves and cuttings from inoculated infected plants using gas train methods and gravi-

metric determination of carbon dioxide. He found respiration of shoots and leaves from diseased plants increased until the first symptoms of wilt appeared.

Fusarinic acid.

Fusarinic acid, a product of the tomato wilt Fusarium (20), has been identified as 5-n-butyl-2-pyridin carboxylic acid (54). Fusarinic acid acts on plants to produce necrotic areas between the leaf veins and streaks on stems of tomato plants. Synthetic fusarinic acid at a concentration of 2×10^{-4} M inhibited respiration of tomato leaf tissue approximately 16 per cent (33). Concentrations greater than 2×10^{-4} M gave more inhibition. The respiration of both healthy and nitrogen deficient tomato plants was decreased after treatment with fusarinic acid but the inhibition was more pronounced in nitrogen deficient plants (57). A similar result has been observed in tobacco leaves treated with fusarinic acid (16).

Physiological effects of ethylene.

The influence of ethylene on fruit respiration has long been known. Lemons exposed to concentrations of ethylene varying from 1 to 1,000 p.p.m. in air had 100 to 200 per cent increase in carbon dioxide evolution (13). Ethylene increased respiration in bananas (19), persimmons (12), pears (24), and plums (47). In 1941 it was shown that citrus fruit infected with Penicillium digitatum (Sacc.) produced a volatile which increased the respiration of uninfected citrus fruits 100 per cent at 15°C. (9). The volatile material produced by P.

digitatum was identified as ethylene (56).

The effects of ethylene on several fruit pectins are known. Ethylene treated pears in early stages of maturity had a more rapid transformation of insoluble protopectin to soluble pectin than had untreated pears (24). This reaction in the presence of ethylene was similar to changes which would normally occur in the more mature fruit. The response to ethylene was slow and lasted a long time. It was noted that respiration and pectins became insensitive to ethylene at approximately the same time. Decreased insoluble protopectin and definite increases in soluble pectin have been reported in the following fruits: gooseberry, peel of ponderosa lemon, Italian prune, Elberta peach, hull of English walnut, and Bartlett and Anjou pears (25). In normally ripening melons insoluble pectin gradually decreased and soluble pectin increased, while in ethylene treated melons increase of soluble pectin was much greater in the early stage of ripening. Since the conversion of protopectin to pectin is caused by "protopectinase" it was thought that ethylene activated this enzyme (37).

The causal role of ethylene in infected plants has been studied in some detail. Rose leaves infected with Diplocarpon rosae (Wolf), cherry leaves infected with Coccomyces hiemalis (Higgins), and chrysanthemum flowers infected with Asochyta chrysanthemi (Stevens) produced more ethylene than did respective healthy tissues as measured by the triple response of etiolated Alaska peas. Injury also caused increased

production of ethylene, since shredded, healthy, rose and cherry leaves produced more ethylene than did uninjured healthy leaves (52). Oranges and grapefruit inoculated with Penicillium digitatum and oranges with Diplodia natalensis (Evans) and Diaporthe citri (Faw.)Wolf) produced epinasty in test pea plants sooner than in normal plants under the same conditions. Diplodia rot in oranges increased after gassing with ethylene but Diaporthe rot was not affected (29). Pure cultures of P. digitatum produced a volatile substance which caused epinasty in treated test plants (32); other pathogens failed to produce ethylene (52).

The production of ethylene in plants infected with Fusarium wilt of tomato was examined by Dimond and Waggoner (14). Ethylene production by infected plants was demonstrated by the amount of epinasty produced in healthy indicator plants confined with the diseased plants. Volatile substances from diseased plants also caused the triple response in peas. It was concluded that ethylene causes the epinastic symptoms associated with Fusarium infected tomato plants. Cultures of Fusarium produced ethylene as measured by the triple response in peas, by epinasty in tomatoes, by bromine addition, and by reversible trapping with mercuric perchlorate. Production of ethylene by the fungus varied with the substrate and appeared to be linked with a heat labile enzyme system, since none was produced by autoclaved cells. Cell free extracts of Fusarium on suitable substrates also liberated ethylene (14).

Permeability changes in infected plants.

Few studies have been conducted on permeability changes in systemically invaded plants. Culture filtrates of F. oxysporum f. lycopersici were reported to cause increased permeability in leaf cells of treated tomato cuttings (45). Since culture filtrates contain many phytotoxic components, evaluation of such measurements is difficult. A better approach is that of Gottlieb (23), who found that tracheal extracts from plants infected with F. oxysporum f. lycopersici affect cell permeability as measured by deplasmolysis time. This reaction was reversible. Sap collected from physiologically wilted plants had little effect on deplasmolysis time. Direct measurements of permeability changes after systemic invasion have not been reported.

MATERIALS AND METHODS

Host material, fungus material, and inoculation methods.

The tomato variety Bonny Best was used in all experiments except those requiring Fusarium resistant plants, in which case the variety Jefferson was used. Plants were grown in sand in the greenhouse and were watered with a balanced nutrient solution (18) or with a suitable concentration of a soluble commercial fertilizer ("Plant Marvel"). Plants from 6 to 8 weeks old were used when cuttings were needed. Stems were cut under water with a razor blade and the cuttings placed in the treating solutions. Cuttings from 8 to 10 inches in length were used.

Fusarium oxysporum f. lycopersici (Sacc.) Snyder and Hansen, strain R5-6, was used throughout (51). The fungus was grown in 500 ml. Erlenmyer flasks in shake culture at approximately 25°C. Under these conditions fungus growth was largely bud cells. The medium of Dimond et al. (14) was used. This medium was formulated as follows:

casamino acids	1.5 gm.
yeast extract	1.0 gm.
KH_2PO_4	1.5 gm.
$MgSO_4 + 7H_2O$	1.0 gm.
glucose	15.0 gm.
trace elements	0.2 ml.
distilled water	to 1000 ml. (pH 5.5 to 5.6).

Plants were inoculated by removing them from the sand,

washing the roots in tap water, and then dipping the roots directly into the bud cell suspension. Plants were then reset in sand and kept under greenhouse conditions favoring disease development. Uninoculated controls in all cases were uprooted, dipped in water, and reset in the same manner as the inoculated plants.

Inoculum for cuttings was prepared by filtering culture fluids through cheesecloth to remove larger particles. The bud cell suspension was centrifuged for 30 minutes at 4410 R.C.F. and the filtrate decanted. The bud cells were resuspended in distilled water, recentrifuged, and then filtered by suction through coarse filter paper to remove aggregates and mycelial fragments. Bud cell suspensions were adjusted to 50,000 spores per milliliter by use of a hemocytometer. Cuttings were placed in these suspensions and allowed to take up bud cells through the cut ends by transpirational pull. Cuttings were then rooted in sand.

After a suitable time for symptom development, plants were rated for wilt and vascular browning. Wilt was rated on an arbitrary basis from 0 to 4; 0 indicated no wilt and 4 indicated severe wilt. The intensity of vascular browning was rated as follows: 0 indicated no vascular browning; 1 indicated slight browning at the base of the stem; 2 indicated browning of the lower 1/3 of the stem; 3 indicated browning of the lower 2/3 of the stem; 4 indicated browning throughout the stem (40).

Methods of determining respiration.

The Warburg apparatus was used to determine oxygen uptake, following in general the method of Klinker (27). Twenty discs were cut from a tomato leaf with a 6 mm. cork borer and placed on moistened filter paper in the bottom of the Warburg flask. Measurements of oxygen uptake were made at 30°C. in a darkened room. Nine Warburg flasks were used in each determination. Four flasks contained leaf discs from inoculated plants, four contained leaf discs from uninoculated plants, and one served as a thermobarometer. The discs in each flask were taken from a single plant. The flasks and their contents were allowed to equilibrate for 30 minutes, after which the stopcocks were closed. Two readings of oxygen uptake were taken at hourly intervals. After measurements of oxygen uptake were completed the leaf discs were removed from the Warburg flasks, dried for 48 hours in a 100°C. oven, and weighed. Oxygen consumption was then expressed as microliters of oxygen uptake per milligram dry weight of tissue or as oxygen uptake per milligram of nitrogen (see later). In other experiments leaf discs were suspended in M/30 potassium phosphate buffer solutions pH 5.9. In these cases leaf discs were vacuum infiltrated with the treating solution for 30 minutes.

To determine respiration in stems, stem slices approximately 0.75 mm. thick were cut using a hand microtome and a straight razor. Slices were taken from the second internode above the cotyledons. The slices were washed in M/30 potas-

sium phosphate buffer at pH 5.9. Stem slices were then removed from the buffer solution, blotted on filter paper, and rapidly transferred to a Warburg flask containing a disc of filter paper moistened with 0.1 ml. of distilled water. Twenty stem slices were used in each flask, and all values were the averages for 3 flasks.

Respiratory quotients were found by the standard method (46). Two flasks were used whose contents were respiring in the same manner. In one the carbon dioxide was absorbed and in the other it was not, giving a measure of the carbon dioxide liberated.

Nitrogen determinations.

In one series of experiments respiration was determined on a nitrogen basis to check the accuracy and validity of expressing respiration in terms of dry weight. Total nitrogen was determined by the micro-Kjeldahl techniques of Ma and Zuazaga (30) and Pepkowitz and Shive (36). Forty to 50 mg. of finely ground dried plant material were placed in a 50 ml. micro-Kjeldahl digestion flask and the contents digested, using concentrated sulfuric acid, until clear. Selenium plus a potassium sulphate-copper sulphate mixture was used as a catalyst. Ammonia was distilled into a flask containing 5 ml. of a 2 per cent boric acid solution. A mixed indicator was added to the boric acid solution and the contents titrated to a pink color with 0.02 N hydrochloric acid. For each determination 24 flasks were used: 12 contained material from healthy plants and 12 contained material from dis-

eased plants.

Method of determining permeability changes.

Conductivity measurements of leachings from healthy and diseased plants were used as a measure of cell permeability. This method measures permeability to electrolytes; the greater the conductivity, the greater the permeability. A conductivity bridge (Model RC16B1 Industrial Instruments, Inc.) was used. Leaves from healthy and diseased plants were carefully selected, washed in double distilled water, and placed in flasks containing 50 ml. of double distilled water. To insure that all leaves were wetted they were either vacuum infiltrated or placed on a reciprocal shaker. After 7 hours the leaves were removed from the water and conductivity readings were made on the water using a conductivity cell with a cell constant of 0.1. All measurements were made at 25°C. Leaves were dried for 48 hours in a 100°C. oven and calculations were made on the basis of conductivity in micromhos per milligram of dry weight of leaf tissue.

Enzyme assays.

Pectin methyl esterase activity in culture filtrates was determined by electrometric titration of free carboxyl groups produced by the action of the enzyme on pectin, as suggested by Gothoskar et al. (21). A 1.5 per cent solution of pectin (pure citrus pectin, N. F., from California Fruit Growers Exchange) was made by slowly adding 15 gm. of pectin to 600 ml. of distilled water using mechanical agitation. To dissolve the pectin the mixture was autoclaved at 15 lb. pressure

for 10 minutes. After cooling, distilled water was added to bring the solution to 1 liter. Ten ml. of a 0.5 M acetate buffer were added to 75 ml. portions of the pectin solution. The culture filtrate was adjusted to pH 7.0 before addition to the pectin buffer mixture. Fifteen ml. of filtrate were added to the pectin buffer mixture. Heated samples of the enzyme and filtrate solution were run as controls. The mixtures were held for 3 hours at 30°C. in a constant temperature bath. The solutions were then titrated to pH 7.0 with a 0.1 N KOH using mechanical stirring. Pectin methyl esterase activity was expressed as "mg methoxyl removed at the end of three hours by one ml of the culture filtrate or one gm of the enzyme sample" (22).

The assay for pectin splitting enzymes (depolymerase and/or polygalacturonase) was based on viscosity measurements. A 0.5 N sodium citrate-citric acid buffer was prepared at pH 4.0 and heated to 50°C. Pectin was added using mechanical agitation until a 1.0 per cent solution was obtained and the solution was passed through several layers of cheesecloth to insure uniformity. Two ml. of culture fluid was added to 20 ml. of the pectin solution and incubated in a water bath at 30°C. for 45 minutes. Five ml. portions of the mixture were placed in an Ostwald viscosimeter and the dropping time noted. Heated culture fluids were used as controls.

Methods of ethylene treatment.

Pure grade ethylene (99.0 mol per cent purity) was obtained from Phillips Petroleum Company. Saturated ethylene

solutions were prepared by bubbling ethylene through distilled water for 30 minutes at 18°C. At 18°C. 100 ml. of water contains approximately 0.015 ml. of ethylene (41). Saturated solutions were diluted with distilled water as needed for the various experiments. Tomato cuttings were allowed to take up these solutions by transpirational pull. In other experiments leaf discs were vacuum ilfiltrated with ethylene solutions. In still other cases plants or cuttings were placed in desiccator jars and gassed directly with measured amounts of ethylene.

Detection of ethylene.

Gas chromatography and infrared spectroscopy were used to detect ethylene in emanations from healthy and diseased plants. For gas chromatography a vapor fractometer (Perkin-Elmer Corp. Model 154) was used to separate and measure the volatile components collected from plants. The volatiles were passed in a stream of gas through a column made of an inactive solid supporting material plus the partition liquid or fixed phase. Different equilibria exist between the mobile phase (carrier gas and sample) and the stationary phase (the column material), causing components to separate according to their individual equilibrium constants.

The concentration of each component was measured by a dual thermocouple and the results were expressed as a series of symmetrical peaks on a recorder. The position of the peak along the ordinate or time axis was used as the qualitative value. The measure of the concentration of a component in

a mixture was the area of the peak or abscissa value (31).

An effort was made to identify ethylene by means of infrared spectrophotometry. Analyses were made using a recording infrared spectrophotometer (Perkin-Elmer Model 21) and an evacuated 4 meter gas cell. This method is based on the fact that nearly all organic substances possess selective absorption at certain frequencies in the infrared portion of the electromagnetic spectrum. The spectrophotometer determines the per cent transmission or absorption of the sample at a series of narrow frequency intervals throughout a selected part of the spectrum. A plot of transmissions or absorption values versus frequency or wave length constitutes the infrared spectrum of the samples (26).

Gases were collected from plant materials in 10 liter desiccators. Four to five kilograms of plant tissue were used in each analysis. Plant materials remained in the desiccator for 2 or 3 days and were supplied with oxygen from a large polyethylene bag connected to the desiccator. Oxygen was added to the bag from an oxygen tank as needed. Gases were removed by vacuum and passed through a series of ascarite and calcium chloride towers to remove carbon dioxide and water. The gases were first collected in a 25 ml. trap immersed in liquid nitrogen. The gases were too dilute to sample directly from the larger trap so they were further concentrated in a 10 ml. trap. This was done by removing the liquid nitrogen, in a Dewar flask, from the larger trap and placing it around the smaller trap. As the larger trap

warmed the gases flowed from it to the smaller trap. Gases were collected from the smaller trap by removing the liquid nitrogen bath and allowing the trap to come to room temperature. Volatiles were then removed from the trap by injecting a needle through a rubber serum bottle cap which was over the side arm. Gases were injected into the vapor fractometer by inserting the needle through a rubber diaphragm into the column. When the gas cell was used volatile materials were injected through a rubber serum bottle cap placed over a small opening in the evacuated cell.

EXPERIMENTAL RESULTS

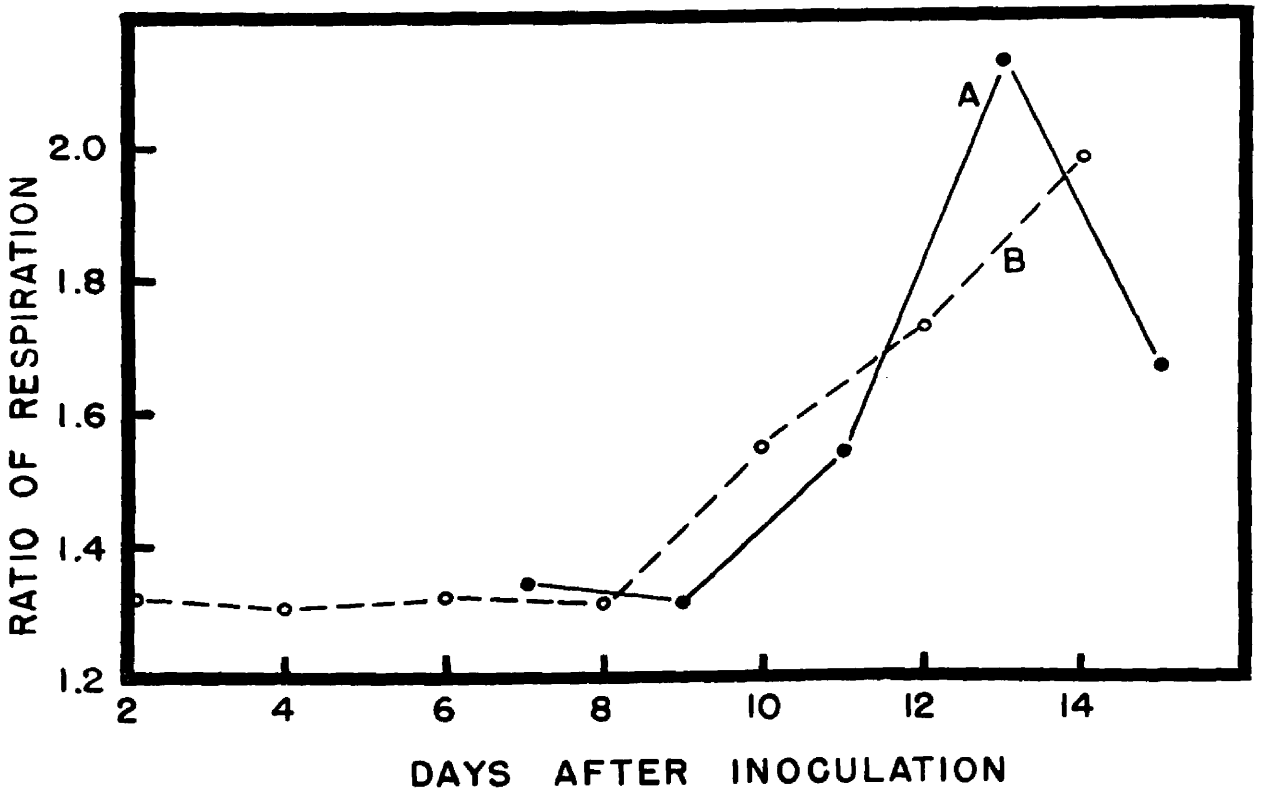
Respiration of leaves from diseased and healthy plants.

Many workers have found increased respiration in leaves directly invaded by strict parasites. Increased respiration has also been found in sound storage tissue adjacent to tissue invaded by certain facultative saprophytes. Other types of diseases, such as vascular wilts, have had little study.

The respiration of leaf discs from healthy and diseased plants was compared following the method of Klinker (27). Plants were inoculated by root dip and kept in the greenhouse until used. For each Warburg determination leaves from 4 inoculated and 4 uninoculated plants were used. The leaves were carefully selected for uniformity and generally the third or fourth leaf from the base of the stem was chosen. Respiration was expressed as oxygen uptake per mg. dry weight or as oxygen uptake per mg. of nitrogen.

In an experiment with plants inoculated 32 days after planting respiratory increases were noted in infected plants 5 days after inoculation. Respiration increased sharply on the 11th day, reached a peak 12 days after inoculation, and dropped off rapidly thereafter (Fig. 1). In a second experiment, 7 weeks old plants were used and a significant increase in respiration was found 2 days after inoculation. Leaves from infected plants had slightly higher respiration for 8 days, but on the 10th day a sharp increase occurred. The respiratory rate continued to climb until the 14th day, when the experiment was ended (Fig. 1).

FIG. 1. Respiration in diseased plants expressed as the ratio of respiration in diseased plants to that in healthy plants under the same conditions, determined on a dry weight basis. Line A. is for plants inoculated 4 weeks after seeding and Line B. is for 7 weeks plants.


RATIO OF RESPIRATION
2.0
1.8
1.6
1.4
1.2
2
4
6
8
10
12
14
DAYS AFTER INOCULATION
A
B

The appearance of diseased and healthy plants used in experiment 2 is shown in Figures 2, 3, and 4. Two days after inoculation there were no differences in appearance of diseased and healthy plants (Fig. 2). Plants 8 days after inoculation (Fig. 3) showed epinasty, slight yellowing of the lower leaves, and stunting. Plants 14 days after inoculation (Fig. 4) were yellowed, stunted, and had some wilting of lower leaves.

Leaf discs from healthy and diseased leaves were made with a sterile cork borer, and the discs were placed on potato dextrose agar, and incubated at 25°C. for 1 week. Fusarium was not isolated from leaves of diseased or healthy plants although repeated attempts were made.

In experiments 1 and 2 respiration was higher in inoculated plants at 2 and 7 days, respectively, when first determinations were made. In a third experiment an effort was made to determine how soon respiration was affected. Significant respiratory increases were noted in diseased plants as early as 1 day after inoculation (Fig. 5). The experiment was ended after 3 days because respiration of plants in later stages of disease had been determined in earlier experiments. This experiment was repeated using washed bud cells as inoculum. The same respiratory pattern was evident, indicating that toxic substances in cultures used as inoculum were not responsible for early respiration increases.

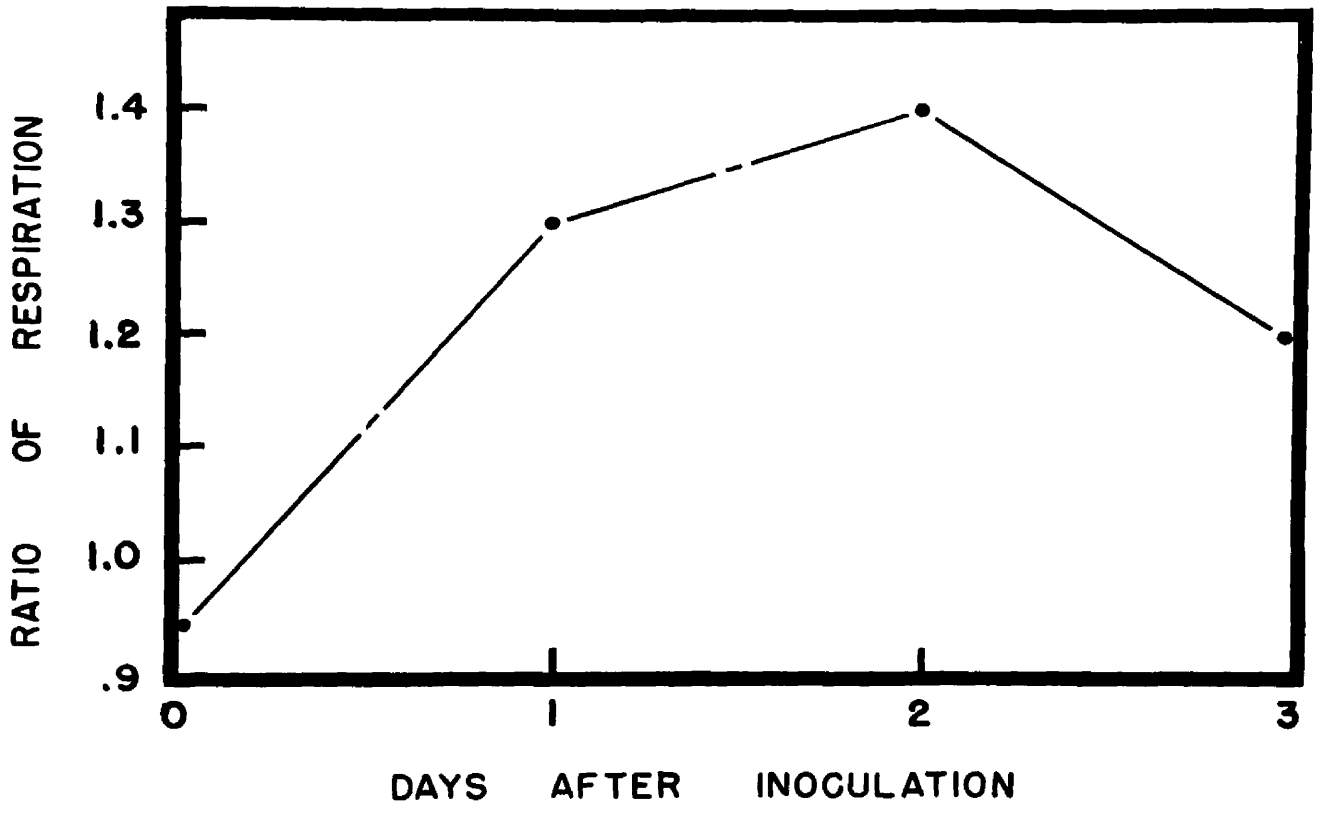
In 2 further experiments respiration of leaf discs from diseased and healthy plants was measured and total nitrogen

FIG. 2. Inoculated plant (right) and uninoculated control (left) 2 days after inoculation.

FIG. 3. Inoculated plant (right) and uninoculated control (left) 8 days after inoculation. Inoculated plant is beginning to show symptoms of disease.

FIG. 4. Inoculated plant (right) and uninoculated control (left) 14 days after inoculation. Inoculated plant showing advanced symptoms of disease.

FIG. 5. Early respiratory changes in infected plants. Respiration in diseased plants is expressed as the ratio with respiration in healthy plants on a dry weight basis.


RATIO OF RESPIRATION
1.4
1.3
1.2
1.1
1.0
.9
0
1
2
3
DAYS AFTER INOCULATION

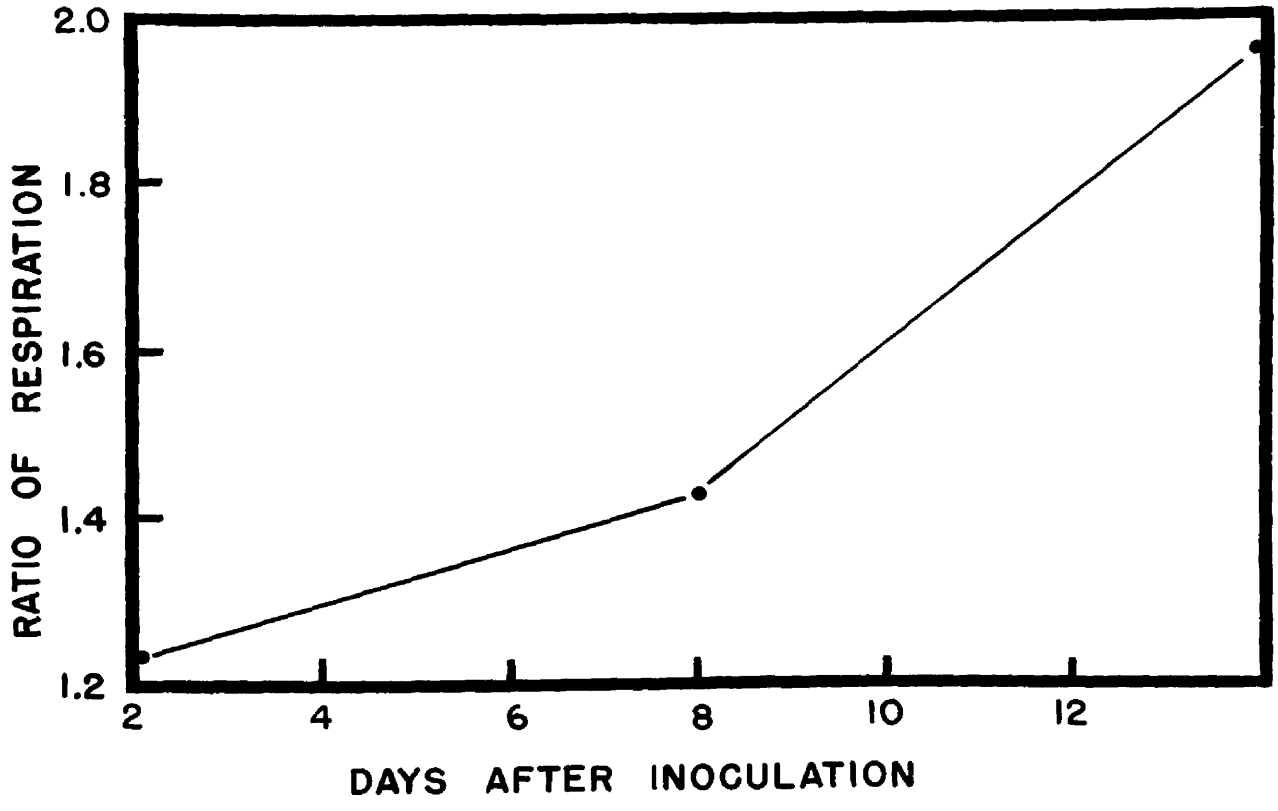
was determined in the leaf samples. Respiration was then expressed as oxygen uptake per mg. of nitrogen. These experiments offered a check on the validity of expressing oxygen uptake on a dry weight basis. Leaves from healthy and diseased plants were collected 2, 4, and 14 days after inoculation. After oxygen uptake was measured, the leaf discs were dried and ground to a fine powder in a Wiley mill, and micro-Kjeldahl determinations were made. Oxygen uptake per unit of total nitrogen again showed that respiration in diseased plants was increased slightly after inoculation, and rose to almost double the value in healthy plants by the 14th day after inoculation (Fig. 6). No differences were found in total nitrogen in plant material collected 2 and 4 days after inoculation but the leaf tissue from diseased plants collected 14 days after inoculation showed a 10 per cent increase in total nitrogen. This difference was not enough, however, to account for the increased respiration of diseased as compared to healthy plants. The experiment was repeated with essentially the same results except that total nitrogen was 18 per cent higher in diseased plants than in healthy plants by the 15th day.

Respiration in stems of inoculated and uninoculated plants.

Respiration of leaves from diseased plants was clearly increased. But what happens to respiration in the stems, where the fungus might be present and fungus products might be in higher concentrations?

Respiration in stems of diseased and healthy plants was

FIG. 6. Respiratory changes in leaves of infected plants determined on a total nitrogen basis. Respiration in infected plants is expressed in relation to respiration in healthy plants.

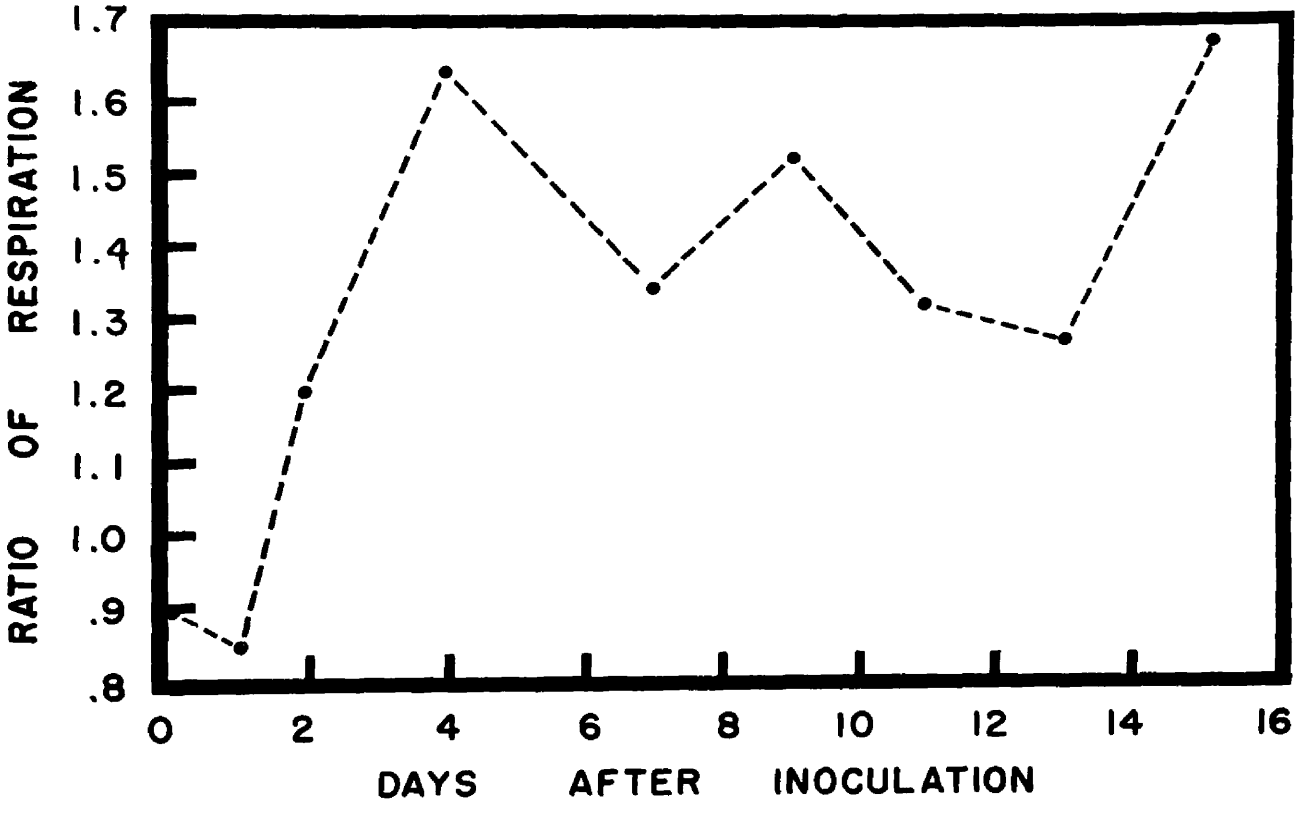

2.0
1.8
1.6
1.4
1.2
RATIO OF RESPIRATION
2
4
6
8
10
12
DAYS AFTER INOCULATION

compared by the use of stem slices, which were taken from the second node above the cotyledons. The stem slices were washed approximately 20 minutes in a M/30 potassium phosphate buffer at pH 5.9. Slices were then removed from the buffer solution, blotted, dry, and transferred to Warburg flasks containing a disc of wet filter paper. This is essentially Klinker's method (27); it was followed after preliminary experiments indicated that it was satisfactory for stem slices.

Four-week-old plants were carefully selected for uniformity. Oxygen uptake by stem slices was determined before inoculation and at 1, 2, 4, 7, 9, 11, 13, and 15 days after inoculation. Respiration dropped slightly 1 day after inoculation, but increased on the second day after inoculation and remained at a higher level than that of the controls throughout the rest of the experiment (Fig. 7). The pattern is similar to that of leaves, except that striking increases may come earlier. Results were more erratic than those obtained with leaves. These experiments were conducted during the winter months when light conditions were poor and root formation was retarded, which resulted in an irregular disease response.

Attempts were made to isolate the fungus from stems of inoculated plants. Stem slices taken from the same region as those used for respiratory measurements were placed on potato dextrose agar and incubated at 25°C. for 1 week. Isolations of the pathogen were made 2 days after inoculation; however, all sections did not contain the fungus until

FIG. 7. Respiration of stem slices from diseased plants expressed as the ratio of respiration in diseased plants to that of healthy plants under the same conditions.


RATIO OF RESPIRATION
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
0
2
4
6
8
10
12
14
16
DAYS AFTER INOCULATION

the 7th day.

Determination of respiratory quotients.

Respiratory quotients of leaves from diseased and healthy plants were determined for comparative purposes. Possible differences might indicate differences in respiratory substrates. Plants 32 days old were inoculated and respiratory quotients were determined 2, 6, 10, and 14 days later, using appropriate controls. Two flasks in each group contained alkali in the center well and two did not. Leaf discs from healthy plants and from diseased plants in all stages of disease development had a respiratory quotient of approximately 1.0 (Table 1). Respiration quotients do not indicate a difference in substrates between diseased and healthy plants.

The effects of fusarinic acid on respiration.

Fusarinic acid, a known product of F. oxysporum f. lycopersici, was tested to see whether or not it could account for the observed increases in respiration. Leaf discs were vacuum infiltrated with solutions of fusarinic acid and M/30 potassium phosphate buffer and oxygen uptake was determined manometrically. In one series concentrations of fusarinic acid at 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, and 10^{-8} M were used at pH 6.0. Respiratory inhibition was noted at 10^{-3} M and at 10^{-4} M. In all other concentrations fusarinic acid had little or no effect on respiration. This experiment was repeated at the same pH but with slightly different concentrations: 10^{-2} M, 5×10^{-4} M, 5×10^{-5} M, 5×10^{-6} M,

TABLE 1. - Respiratory quotients of leaf discs from from healthy and diseased tomato plants.

Days after inoculation	Respiratory quotients (a)	
	Healthy	Diseased
2	0.92	0.95
4	0.95	0.96
6	0.97	0.94
10	0.95	0.93

(a) Average of 4 Warburg determinations.

5×10^{-7} M, and 5×10^{-8} M. In this experiment fusarinic acid at 10^{-2} M markedly inhibited respiration. At 5×10^{-4} M there was slight inhibition, but lower concentrations were without effect. In a third series, fusarinic acid was used at 10^{-3} M, 5×10^{-4} M, 5×10^{-5} M, 10^{-6} M, 10^{-7} M, and 10^{-8} M at pH 4.8. Respiration was inhibited in concentrations of 10^{-3} M and 5×10^{-4} M, but was not significantly affected at the other concentrations (Table 2). It was concluded that fusarinic acid was not responsible for respiratory increases in infected plants.

Respiration of pectinase treated cuttings.

Since Fusarium produces pectic enzymes, an experiment was designed to test the effect of pectolytic enzymes on respiration. Pectinase, a mixture of pectolytic enzymes, was used. Tomato cuttings were allowed to take up solutions of pectinase for 3 days following the procedure of Scheffer and Walker (39). Leaf discs were then cut and respiration was determined manometrically. Active pectinase in 0.5, 0.25, and 0.125 per cent solutions along with appropriate heated controls were used. All levels were run with 5 cuttings each, Pectinase had no effect on respiration in any of the concentrations used. It was concluded that pectinase was not responsible for the respiratory increases in infected plants.

Effects of ethylene on respiration of leaves and stems.

Ethylene is known to increase respiration in certain fruits (24), but its effect on leaves has never been examined. Since ethylene is a known product of Fusarium infected

TABLE 2. - The effect of fusarinic acid on respiration of tomato leaf discs.

Experiment No. and pH	Molar Concentration	Average (a) l O_2/mg. dry wt./hr.	Respiratory ratio (Expressed as per cent of control)
1 pH 6.0	control	2.58	--
	10^{-3} M	2.29	0.88%
	10^{-4} M	2.50	0.96%
	10^{-5} M	2.29	0.88%
	control	2.16	--
	10^{-6} M	2.15	0.99%
	10^{-7} M	1.94	0.90%
	10^{-8} M	2.07	0.96%
2 pH 6.0	control	2.45	--
	10^{-2} M	1.10	0.45%
	10^{-4} M	2.13	0.87%
	10^{-5} M	2.42	0.99%
	control	2.44	--
	5×10^{-6} M	2.51	1.03%
	5×10^{-7} M	2.25	0.92%
	5×10^{-8} M	2.72	1.11%
3 pH 4.8	control	3.59	--
	10^{-3} M	2.54	0.71%
	5×10^{-4} M	3.05	0.85%
	5×10^{-5} M	3.54	0.98%
	control	2.61	--
	10^{-6} M	2.53	0.97%
	10^{-7} M	2.73	1.04%
	10^{-8} M	2.84	1.08%

(a) Average reading for two flasks.

tomato plants (14) the possibility that it may account for observed respiratory increases became apparent.

The effect of ethylene on the respiration of healthy tomato plants was tested in several ways. In one series of experiments cuttings from 8 to 10 inches in length were allowed to take up ethylene solutions which were 1:10, 1:100, 1:500, and 1:1000 dilutions of a solution saturated at 18°C. Cuttings were treated with ethylene for 3 days. The solutions were changed and the bases of the stems trimmed each day. These experiments were done in the laboratory under artificial light at approximately 24°C. For each concentration 5 flasks each containing 1 cutting were used. Five flasks each containing distilled water and a cutting served as a control. After 3 days treatment, leaf discs and stem slices were cut and oxygen consumption was measured manometrically. Results showed that ethylene did not change respiratory rates in stems or cuttings.

The respiration of leaves from 25 days old plants gassed with ethylene was determined. Plants were placed in 10 liter vacuum desiccators and concentrations of ethylene in air of 1:10 and 1:1000 were added to the partially evacuated desiccator by injecting a needle through a rubber serum bottle cap placed over the opening in the side arm of the desiccator. The same method was used to fill the syringe from the ethylene tank. The desiccators were allowed to come back to atmospheric pressure, resealed, and plants were held overnight. Results showed that oxygen uptake by leaf discs from gassed

plants did not differ from oxygen uptake by untreated controls.

In 4 similar experiments excised leaves from 5-weeks-old tomato plants were gassed with 1:10, 1:100, 1:500, and 1:1000 ethylene-air mixtures. Two leaflets taken from each of 5 plants were used for each mixture. Controls were treated the same way except that ethylene was omitted. The leaflets were left 12 hours in the gas chambers before discs were cut and oxygen uptake determined manometrically. Results again showed no effect of ethylene on leaf respiration.

Leaf discs from 35-days-old tomato plants were vacuum infiltrated with ethylene dissolved in M/30 potassium phosphate buffer solutions at pH 5.9. Ethylene saturated solutions were diluted 1:10, 1:100, and 1:1000. After 1 hour in the solutions, leaf discs were removed, blotted dry, and quickly transferred to Warburg flasks. Results showed no effect of the ethylene solutions on oxygen uptake by leaf discs. It was concluded that ethylene was not responsible for the respiratory increases in diseased plants.

Effect of ethylene on disease development.

Inoculated and uninoculated plants of resistant and susceptible tomato varieties were treated with ethylene to determine whether or not ethylene could alter the course of disease, such as is caused by ethanol and several respiration inhibitors (21). Cuttings were allowed to take up bud cell suspensions by transpirational pull. The following day cuttings were placed in solutions of ethylene prepared by diluting a saturated solution 1:10 and 1:100 with tap water. Cut-

tings were kept in ethylene solutions, with frequent changes of solution, for 3 days. Appropriate controls were used, including inoculated and untreated plants, uninoculated and ethylene treated plants, and uninoculated and untreated plants.

When the cuttings developed roots they were potted in sand and kept under conditions favorable for disease development. Five plants were used in each treatment. Fourteen days after inoculation plants were rated for severity of wilt and vascular browning. Results showed that disease developed more rapidly in ethylene treated, inoculated plants than in the inoculated untreated controls of the susceptible variety. The uninoculated treated controls showed non-permanent epinasty and slight stunting but no other response similar to that of diseased plants. At the end of the experiment there were no evident differences between uninoculated treated plants and uninoculated untreated plants.

The effect of ethylene on expression of resistance by a resistant variety was determined. Cuttings were inoculated and treated with a saturated solution or a 1:5 dilution of ethylene for 6 days and rooted in sand. Fourteen days later inoculated treated cuttings showed typical symptoms of disease. Inoculated untreated controls gave no evidence of disease. Uninoculated treated controls showed epinasty and slight yellowing of lower leaves and were slightly stunted at the end of the experiment. No other response similar to diseased plants was noted.

Attempts were made to isolate the fungus from inoculated

treated plants by placing stem slices on potato dextrose agar. Positive isolations were obtained along the entire length of inoculated treated plants but were made only from the basal portion of inoculated untreated plants.

Other possible effects of ethylene.

Ethylene was reported by Hansen (25) to make pectin more soluble and Rosa (37) reported that ethylene activated pectolytic enzymes. Since pectic enzymes are involved in Fusarium wilt (13), the possibility of ethylene-pectin and ethylene-enzyme interactions was considered.

To determine whether or not ethylene acted upon the pectin directly, ethylene was bubbled through a 1 per cent pectin solution for 30 minutes at 18°C. Viscosity measurements were made on the pectin-ethylene mixture after standing for various periods of time (1 hr., 6 hrs., 12 hrs., and 24 hrs.). The viscosity measurements showed ethylene to have no direct effect upon this particular pectin under the conditions of these experiments.

The possible effect of ethylene on pectin methyl esterase was determined. Fusarium was grown in wheat bran cultures for 5 days, after which 200 ml. of water were added to each flask and the cultures left to autolyze for 12 hours. The autolyzed wheat bran was squeezed through cheesecloth and clarified by centrifugation at 4410 R.C.F. for 15 minutes. Ethylene was bubbled through the clarified solution until it was saturated and the solution was then assayed for pectin methyl esterase activity using the standard procedure. Ethyl-

ene had no effect on pectin methyl esterase activity.

Viscosity measurements were used to determine the effect of ethylene on pectin splitting enzymes from Fusarium. The fungus was grown as outlined above and ethylene was bubbled through the clarified solution. Depolymerase activity as measured by reduction in viscosity was not affected by ethylene.

Detection of ethylene.

Dimond and Waggoner (14) have shown that ethylene is produced by both Fusarium and Fusarium infected plants. Many methods used to identify ethylene are non-specific and unsaturated compounds might give a positive reaction. Infrared spectroscopy and gas chromatography were used because they are specific and also can yield a complete spectrum of volatiles from plants. Ethylene was not positively identified as a volatile material from diseased plants due to the great difficulty in separating mixtures recorded on the infrared spectrograph. Figure 8 shows an infrared spectrograph of gases collected from diseased plants. It can be seen that some of the peaks in the unknown correspond to peaks produced by pure ethylene (Fig. 9). One component which was positively identified by both gas chromatography and infrared spectroscopy was nitrous oxide (laughing gas) (Figs. 10, 11). However, nitrous oxide was not an emanation from either the fungus or diseased plant material, but apparently came from a bacterial contaminant which grew on the leaves as they collapsed.

FIG. 8. Infrared spectrograph of gases collected from diseased plants.

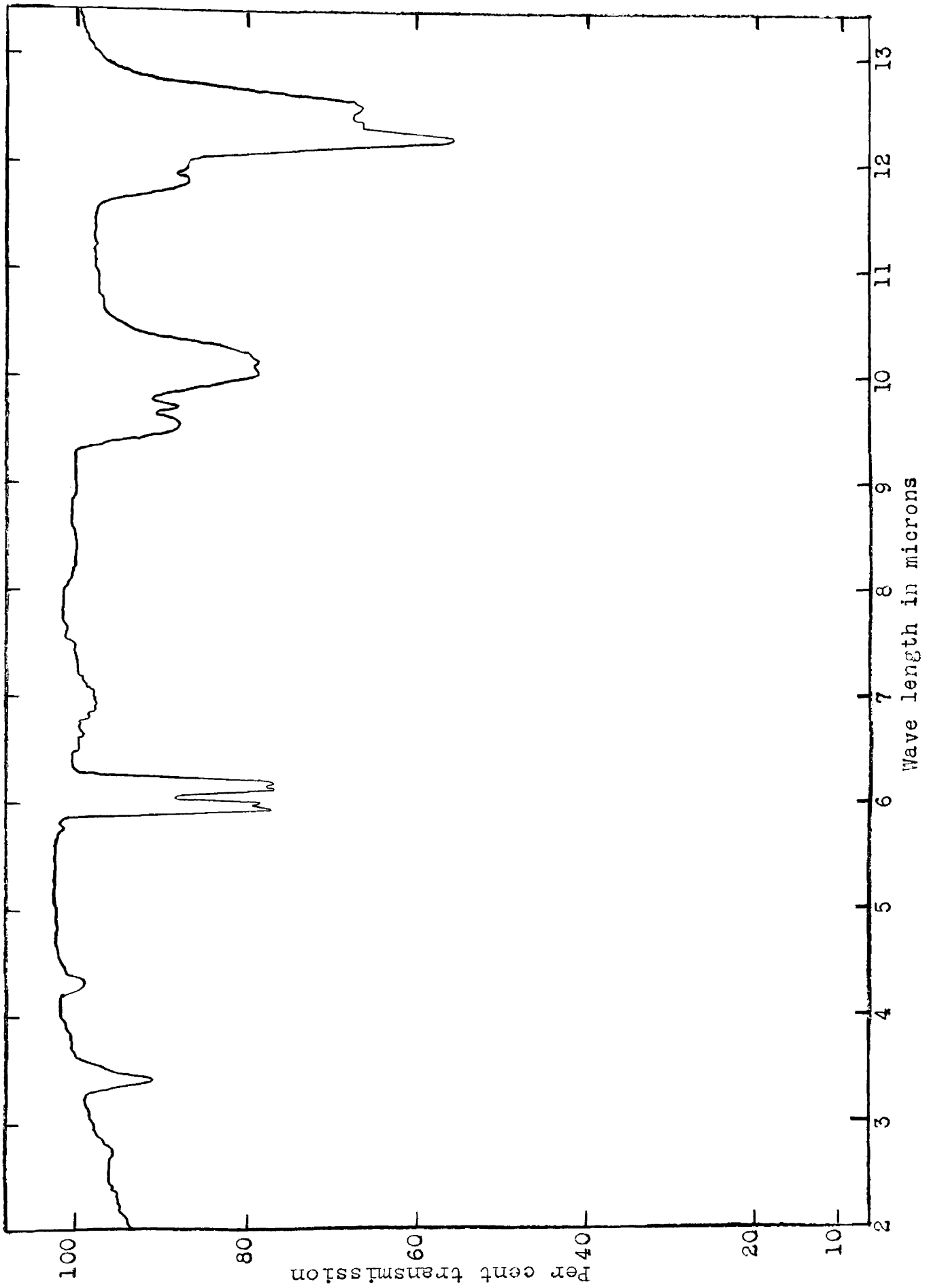

Per cent transmission
100
80
60
40
20
10
2
3
4
5
6
7
8
9
10
11
12
13
Wave length in microns

FIG. 9. Infrared spectrograph of pure ethylene.

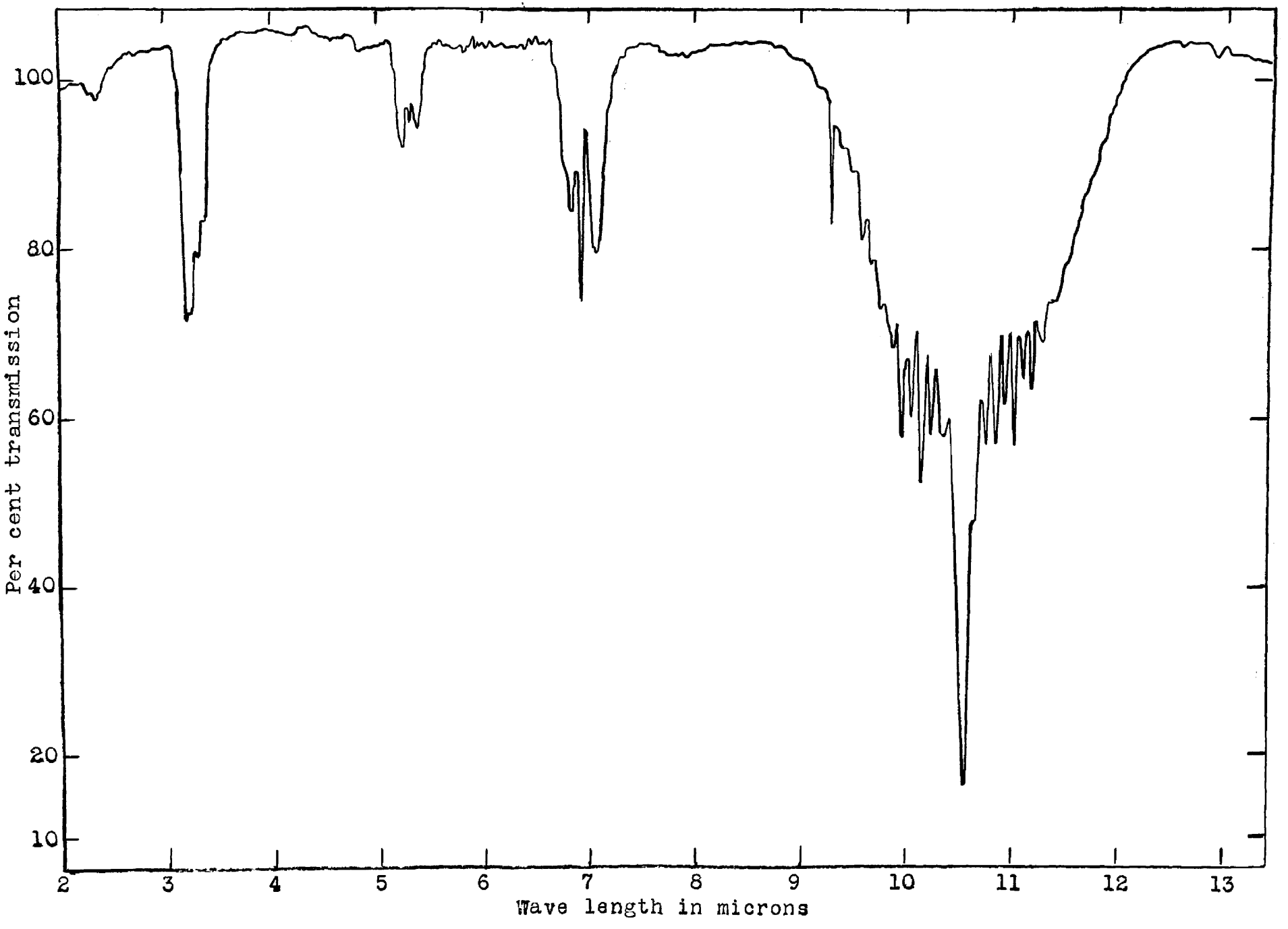

100
80
60
40
20
10
Per cent transmission
2
3
4
5
6
7
8
9
10
11
12
13
Wave length in microns

FIG. 10. Infrared spectrograph showing nitrous oxide in gases from tomato plants.

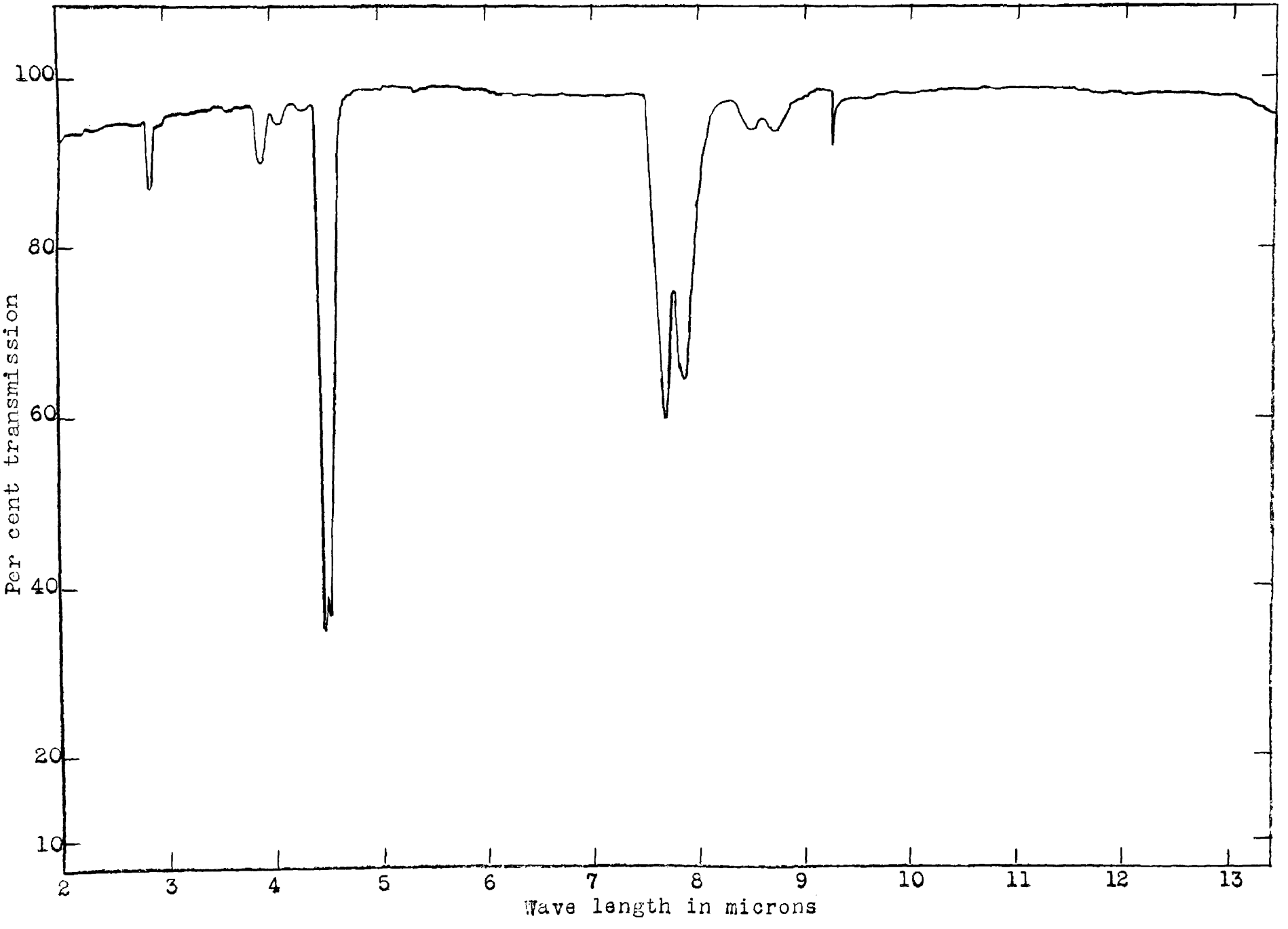
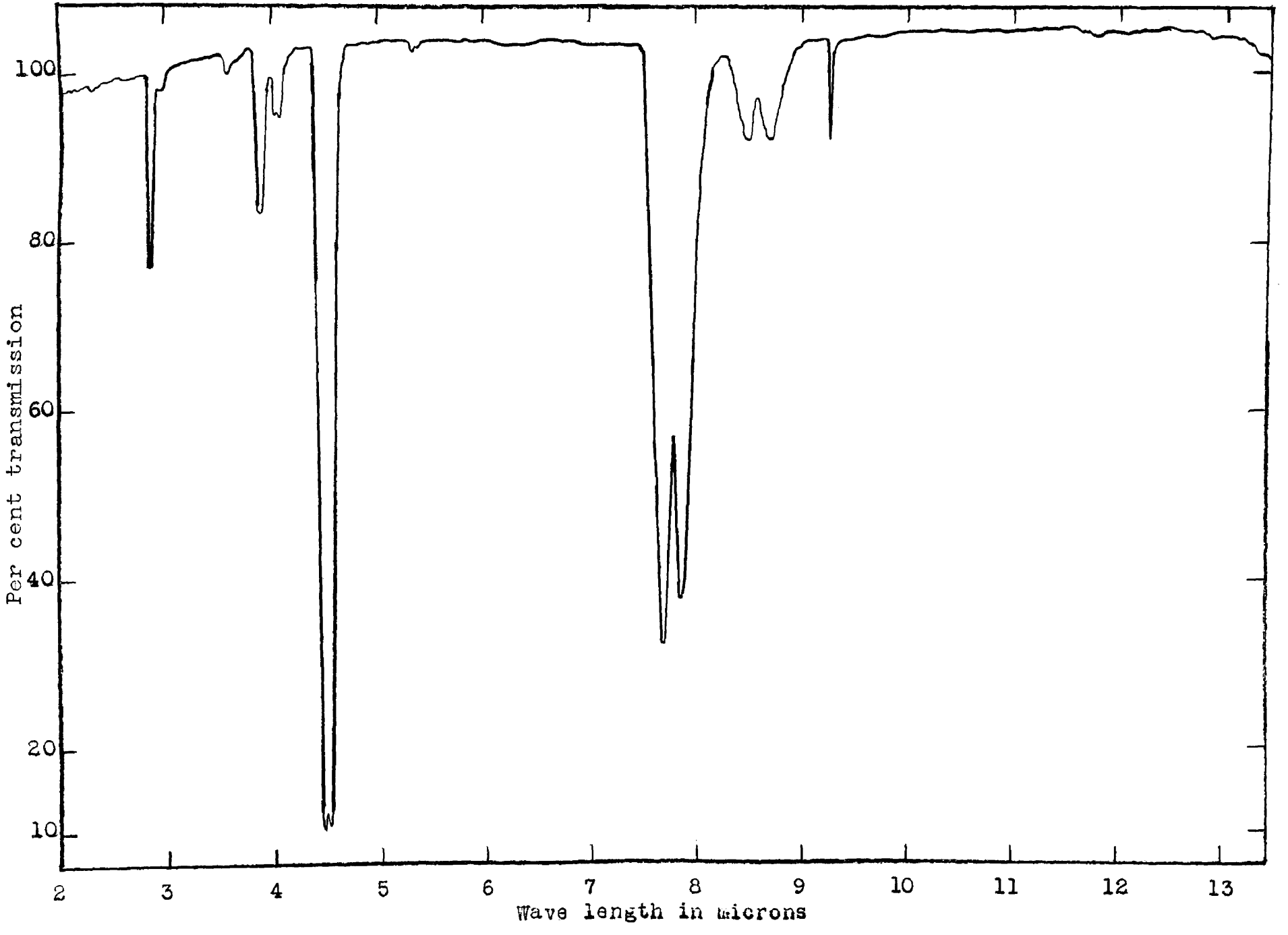

100
80
60
40
20
10
Per cent transmission
2
3
4
5
6
7
8
9
10
11
12
13
Wave length in microns

FIG. 11. Infrared spectrograph of pure nitrous oxide.


100
80
60
40
20
10
Per cent transmission
2
3
4
5
6
7
8
9
10
11
12
13
Wave length in microns

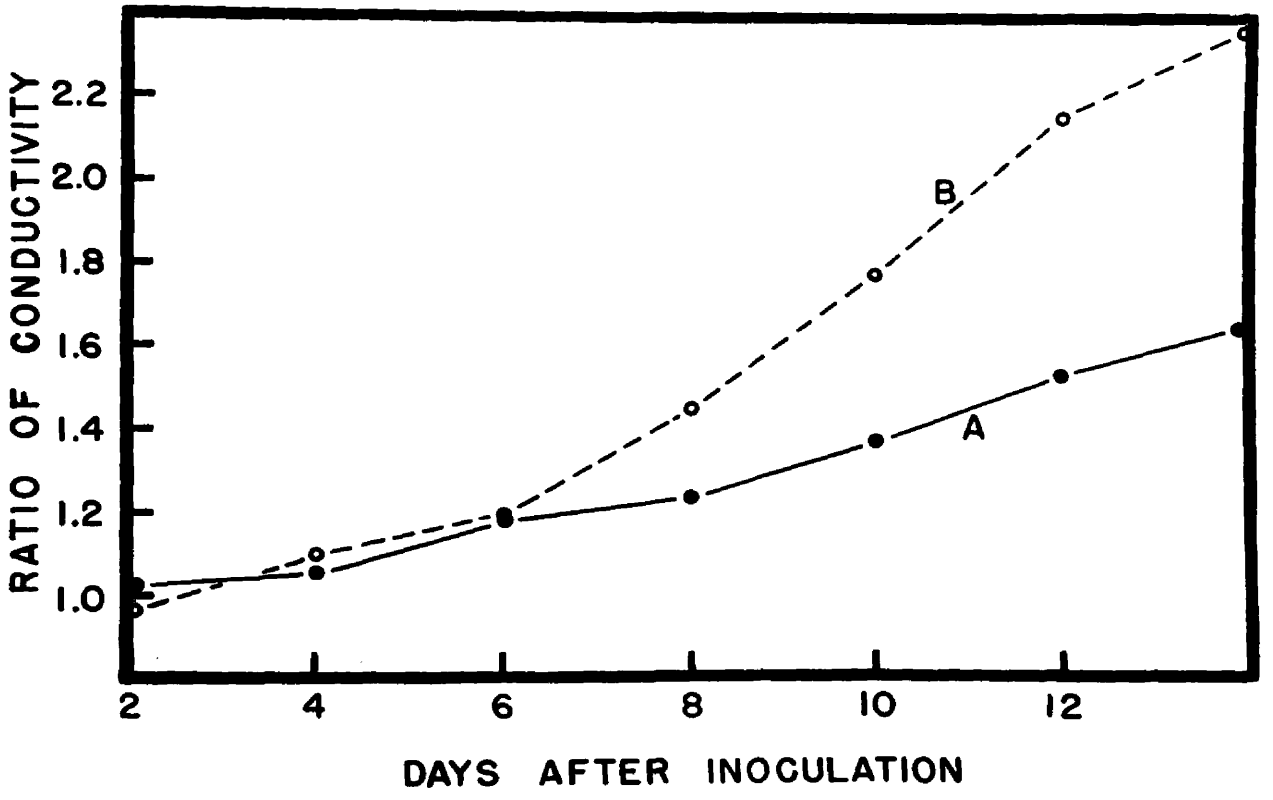
The nitrous oxide peaks as shown by both infrared spectroscopy and gas chromatography were so pronounced that tests were conducted to see whether or not nitrous oxide could cause the epinastic response characteristic of ethylene. Plants were placed in 10 liter vacuum desiccators and gassed with nitrous oxide using the needle and syringe method previously described. Nitrous oxide at concentrations of 1:10, 1:100, and 1:1000 caused epinasty. However, when cuttings were allowed to take up solutions of nitrous oxide no epinasty resulted.

Conductivity studies.

Conductivity measurements of leachates from leaves of healthy and diseased plants were made to determine whether or not infection affected tissue permeability. The method measures the permeability of cells to electrolytes; the greater the conductivity, the greater the permeability. Plants used in these experiments were grown in the greenhouse, inoculated by the root-dip method, and kept under conditions favoring disease. Experiments were designed so that readings were taken at intervals as disease developed. In each experiment 30 inoculated plants and 30 uninoculated plants were used. An upper leaflet and a lower leaflet were taken from each plant, and 5 leaflets were placed in each flask containing 50 ml. of double distilled water. Appropriate controls of double distilled water were included. After 7 hours in the water leaves were removed and conductivity of the water was measured. Results showed that infection caused increased

permeability of leaf tissue beginning 4 days after inoculation and increasing until advanced symptoms were apparent (Fig. 12). This held true for both upper and lower leaves. This experiment was repeated twice.

FIG. 12. Conductivity of leachates from diseased plants expressed as the ratio of conductivity of leachates from diseased plants to that from healthy plants.


RATIO OF CONDUCTIVITY
2.2
2.0
1.8
1.6
1.4
1.2
1.0
B
A
2
4
6
8
10
12
DAYS AFTER INOCULATION

DISCUSSION

Increased respiration has been reported in plants infected with strict parasites and with facultative saprophytes. With few exceptions the respiratory picture has been complicated by the difficulty in separating the parasite from the host tissues. Many studies, however, have indicated that a diffusible substance must be present because increased respiration was found in areas where the fungus had not penetrated. Fusarium wilt of tomato is ideal for studies of pathological metabolism and toxin production in vivo because affected host tissue free of the pathogen is easily obtained.

The respiration of leaf discs from inoculated and uninoculated tomato plants was compared, and it was found that respiration of inoculated plants significantly increased as early as 1 day after inoculation. The most pronounced changes in respiration occurred 12 to 14 days after inoculation. The respiratory curve appeared to consist of two phases, with an increase in respiration 1 day after inoculation and again a marked increase 12 to 14 days after inoculation. Between these peaks respiration though consistently higher than the controls remained at a relatively stable level. This suggests that 2 reactions or series of reactions may be involved. The first may be the more sensitive, and is affected shortly after inoculation. When toxins become more concentrated, or when other toxins enter the picture, the second rise in respiration may occur.

Attempted isolations from leaf blades of inoculated plants were always negative, indicating that a diffusible substance must be present to account for respiratory responses in leaves.

Several substances thought to be involved in Fusarium wilt were examined to see if they were responsible for the increased respiration. Ethylene, a known vivotoxin, was thoroughly tested for any possible effects on leaf or stem respiration. Leaf discs were vacuum infiltrated with various concentrations of ethylene and oxygen uptake was determined. Cuttings were allowed to take up various concentrations of ethylene by transpirational pull and respiration of leaves was determined. Whole plants placed in desiccators were gassed with ethylene and respiration of leaf discs and stem slices was determined. Results in all cases showed that ethylene did not affect respiration, which indicates that it is not the cause of respiratory disturbances in infected plants.

Because pectolytic enzymes are involved in Fusarium wilt of tomato, tests were made to determine whether or not pectinase, a commercial enzyme preparation, had any effect on respiration. The respiration of leaf discs taken from treated cuttings was unaffected by pectinase. More experiments should be conducted using purified enzyme preparations before the pectolytic enzymes are excluded as respiratory toxins.

Fusarinic acid, a product of the tomato wilt Fusarium, has been shown by Naef-Roth and Reusser (33) to be a respir-

atory inhibitor at concentrations of 10^{-4} M and above. They did not, however, report the effect of more dilute solutions of fusarinic acid on respiration. The inhibitory effect of fusarinic acid on respiration reported by Naef-Roth and Reusser was confirmed but at lower concentrations (10^{-5} M to 10^{-8} M) there was no effect on respiration. These experiments indicated that fusarinic acid was not responsible for observed respiratory disturbances in infected plants. Furthermore, fusarinic acid is a necrotic agent, and since necrosis was seldom seen, this or similar toxins may be of little significance in the disease syndrome.

It could be argued that respiration based on a dry weight basis might give erroneous results if there were great differences in the amount of protoplasm present in inoculated as compared to uninoculated plants. Respiration on a nitrogen basis was determined, and although significant increases in total nitrogen were found for plants in the terminal stages of disease, the increases were not great enough to account for the increased respiration. The type of nitrogenous substance accumulating was not determined.

Nitrogen changes have been found in sweet potatoes infected with Ceratostomella fimbriata (8). In sound tissue adjacent to infected areas mitochondrial enzymes ("functional protein") are increased. Decrease in acid soluble nitrogen and increase in acid insoluble nitrogen have also been reported (7). Nitrogen changes associated with Fusarium wilt may follow this pattern.

The effect of environment on respiration was not systematically studied but some observations were made. Allen (3) found that wheat plants infected with powdery mildew had decreased respiration in the winter months and an increase in summer. He attributed this reduction to a lack of sunlight. The respiration of tomato plants inoculated with Fusarium also differed from summer to winter. In general the respiration was lower and more erratic during the winter months. The reasons for this are not understood but several factors which may play a role are light, temperature, and root formation. The last is probably very important for if the plants have few roots and develop new ones slowly, systemic invasion by the fungus is retarded. Such plants also form roots more slowly. Certain nutritional factors could have an effect similar to that of light.

The respiratory pattern of stem slices from inoculated and uninoculated plants followed that of the leaf discs. Stems, however, were less satisfactory for studying respiration because the fungus was undoubtedly present and because results were more erratic. The increased respiration in stems would appear to be more than the additive effect of the fungus. The pathogen is confined to xylem vessels, which are a very small proportion of the whole plant (39). From this evidence it seems apparent that the increased respiration is not due to the presence of the fungus alone. More direct evidence was found when the isolation data were examined. Although a marked respiratory increase was found

as early as four days after inoculation and positive isolations of the fungus were made two days after inoculation, it was not until seven days after inoculation that all sections contained the fungus.

Permeability changes have been used as an indication of toxic action and pathological changes in infected plants. Gottlieb (23) has shown that young tomato plants treated with tracheal extracts from diseased plants have an increased permeability. He felt that the increased permeability was due to a toxin. Culture filtrates of F. oxysporum f. lycopersici caused increased permeability of leaf cells according to Thatcher (45). Thatcher did not test for permeability changes in infected plants.

Permeability changes were measured in the present work by conductivity tests of leachates from diseased and healthy plants. Results showed that permeability of leaf blade cells was increased by disease as early as 4 days after inoculation, and continued to rise until advanced symptoms appeared. The changes in permeability may parallel those found in respiration experiments. Any change in respiration may affect permeability, and any change in permeability may affect respiration.

Another approach, which was not used in this study, is the one reported by Linskens (28). He determined the effect of fusarinic acid on the permeability of tomato leaves using conductivity methods and found substantial increases. Leachates were examined using chromatographic techniques and it

was found that they contained not only various ions but amino acids and other proteinaceous substances normally found in the interior of cells. Linskens concluded that fusarinic acid gave rise to a true change in permeability since the changes were found in non-necrotic leaves and the substances found were characteristic intracellular substances. Linskens' data are not to be taken as proving that fusarinic acid is a significant factor in disease.

An attempt was made to determine the role of ethylene in disease. It has already been pointed out that ethylene had no effect on respiration. The conversion of insoluble pectin to soluble pectin by ethylene has been reported in the literature. It has also been reported that ethylene increases the activity of pectolytic enzymes. In these experiments ethylene had no direct effect upon pectin used nor did it increase the activity of pectolytic enzymes tested.

Dimond and Waggoner (14) have shown that ethylene gives rise to certain symptoms associated with Fusarium wilt of tomato. How ethylene acts to bring about these changes is unknown. In this study the observations reported by Dimond and Waggoner were confirmed and other effects of ethylene were noted. Disease development was hastened in inoculated cuttings of a susceptible variety treated with ethylene. Ethylene caused inoculated, treated plants of a resistant variety to develop typical Fusarium wilt symptoms. The observation was confirmed by isolating the causal organism from along the entire length of the stem.

Other substances which are known to alter resistance are sodium diethyl dithiocarbamate, thiourea, sodium fluoride and 2-4 dinitrophenol. These compounds are interesting in that they all block certain enzymatic reactions in the glycolytic and tricarboxylic schemes (21). Ethyl alcohol also produces typical Fusarium wilt symptoms in resistant inoculated plants; it also may interfere with respiration and cause decreased permeability of cell membranes. How ethylene acts to break resistance is not known but it does not appear to act via respiratory mechanisms.

Ethylene has been detected from cultures of Fusarium and from diseased and healthy plants according to Dimond and Waggoner (14). The methods used by these workers would undoubtedly detect ethylene, but they might also detect other unsaturated compounds. For this reason and because emanations from plants have been little studied gases from healthy and diseased plants were analyzed.

Infrared spectroscopy and gas chromatography were tried. As yet the work is inconclusive, the greatest difficulty being sampling and characterization of mixtures. A number of useful techniques have been developed, and with time a complete spectrum of volatiles from diseased plants should be possible. One volatile which occurred as a bacterial contaminant in a desiccator containing plant materials was nitrous oxide. Nitrous oxide was shown to elicit an epinastic response, the significance of which is unknown. Nitrous oxide has been reported before as a product of denitrifying bac-

teria (1, 49). Methods of gas chromatography and infrared spectroscopy may help in determining if nitrous oxide represents a normal intermediate in the conversion of nitrate to nitrogen or if it lies outside the regular pathway of nitrate reduction.

SUMMARY

Respiration of leaf discs taken from diseased plants was significantly higher than leaf discs from uninoculated controls as early as 1 day after inoculation. The peak of respiration in diseased plants was reached 12 to 14 days after inoculation, when it was sometimes double that of the controls. This relationship held whether respiration was determined on a dry weight basis or on a nitrogen basis. A similar pattern was evident in stems, as determined manometrically using stem slices. The pathogen could not be isolated from leaf blades of infected plants, indicating that a systemic toxin was involved. The pathogen was present in the lower stem as early as 2 days after inoculation but all stem sections used for determination of respiration did not contain the pathogen until 7 days after inoculation.

No changes in total nitrogen were found 2 or 8 days after inoculation but on the 14th and 15th days leaf tissue from diseased plants showed 10 to 18 per cent increase in total nitrogen.

Various substances known to be produced by F. oxysporum f. lycopersici were tested for their effect on host respiration. Fusarinic acid at concentrations of 10^{-2} M, 10^{-3} M, and 10^{-4} M had an inhibitory effect on leaf respiration, but in lower concentrations it was without effect. Pectinase, a commercial enzyme preparation consisting of several pectolytic enzymes, had no effect on respiration of leaf discs

taken from treated cuttings regardless of the concentration used. Ethylene was suspected as the cause of respiration increases. Cuttings were allowed to take up ethylene solutions by transpirational pull and respiration of leaves was determined. In other experiments leaf discs were vacuum infiltrated with ethylene solutions, and in still other cases leaves were gassed. In no case did ethylene affect leaf respiration.

Ethylene treatment of inoculated cuttings hastened disease development in susceptible tomato plants. When applied over a 6 day period to plants of a resistant variety, typical disease symptoms resulted. Using standard assay methods ethylene was shown to have no effect on pectin or pectic enzymes from Fusarium.

The permeability of diseased plants as measured by conductivity of leaf leachates was significantly increased 4 days after inoculation. Permeability thus determined increased as disease developed, until by the 12th day it was approximately double that of controls.

Gas chromatography and infrared spectroscopy were used in attempts to identify ethylene as an emanation from diseased plants. Results were inconclusive. A bacterial contaminant growing on tomato leaf tissue produced an emanation identified as nitrous oxide.

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