Meloidogyne incognita (nematode) parasitism of *Lycopersicon esculentum* (tomato) plants. Ethylene action in susceptible and resistant host responses

David Baxter Akitt, B.Sc.

Department of Biological Sciences

A thesis

submitted in partial fulfillment

of the requirements for the degree of

Master of Science

September 1978

Brock University

St. Catharines, Ontario

This work is dedicated to the late

Jacob Schick

my grandfather and an inspiration

ABSTRACT

AKITT, DAVID B. Meloidogyne incognita (nematode) parasitism of Lycopersicon esculentum (tomato) plants. Ethylene action in susceptible and resistant host responses.

Involvement of ethylene in the etiology of tomato plants (Lycopersicon esculentum) infected with the root-knot nematode (Meloidogyne incognita) was investigated. Endogenous root concentrations of ethylene were not significantly different in uninfected resistant var. Anahu and susceptible var. Vendor plants. Exposure of resistant plants to high doses of infectious nematode larvae did not affect root ethylene concentrations during the subsequent 30 day period. The possibility that ethylene may be involved in the mechanism of resistance is therefore not supported by these experiments. In no experiments did ethylene concentrations in roots of susceptible plants increase significantly subsequent to M. incognita infestation. This result is not consistent with the hypothesis in the literature which suggests that increased ethylene production accompanies gall formation.

Growth of susceptible tomato plants was affected by M. incognita infestation such that root weights increased (due to galling), stem heights decreased and top weights increased. The possibility that alterations in stem growth resulted from increased production of 'stress' ethylene is discussed. Growth of resistant plants was unaffected by exposure to high doses of M. incognita and galls were never detected on the roots of these plants.

Root ethane concentrations generally varied in parallel with root ethylene concentrations although ethane concentrations were without exception greater. In 4 of 6 experiments conducted ethane/ethylene ratios increased significantly with time. These results are discussed in the light of published data on the relationship between ethane and ethylene synthesis.

The term infested is used throughout this thesis in reference to plants whose root systems had been exposed to nematodes and does not distinguish between the susceptible and resistant response.

ACKNOWLEDGEMENTS

I would like to extend grateful appreciation to Dr. Alan Bown for his continued support, constructive critisims and guidance over the course of this thesis. I am grateful to Dr. Frank Marks for his role in the inception of this work and to Dr. John Potter for abundant assistance as a member of my supervisory committee. Thanks are also due to Dr. Peter Nicholls who chaired and organized committee meetings and to Dr. A. J. McGuinnes, Director of the Canada Department of Agriculture Research Centre at Vineland Station, Ontario, for use of headerhouse and greenhouse facilities.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

The host-parasite interrelationship of root-knot nematodes on the roots of suitable host plants is a highly specialized and complex association. According to Mountain (1960a) the genera Heterodera and Meloidogyne are the most highly evolved plant parasitic nematodes as indicated by their advanced degree of morphological and physiological specialization. They are obligate parasites (Thorne 1961) with well defined physiologic and morphologic alterations in their hosts and may be considered pathogens (Mountain 1960b). Species of the genus Meloidogyne characteristically induce the formation of galls on host roots. These arise from intense mitotic activity of the parenchyma cells surrounding the parasite which usually lies in the vascular tissue. Also associated with Meloidogyne infestation is the formation of giant cells or syncytia which are large multinucleate cells of great metabolic activity about the head of the nematode and on which the parasite feeds. The morphologic and physiologic alterations are reviewed in detail in the next section.

The highly specific nature of this host-parasite relationship has resulted in voluminous research aimed at explaining the etiology in biochemical terms. Considerable advances have been made in describing the changes in enzyme and hormone concentrations within the modified host cells. Investigations of the latter have centred around auxins, gibberellins and cytokinins. Recently, however, the hormone ethylene has been implicated in gall formation. Root-knot nematode infested plants often show signs of senescence including yellowing and abscission of leaves, stunting of stem

growth and epinasty (Christie, 1936, Orion and Minz 1969, Orion 1973). These symptoms can be induced in healthy tissue by exogenous ethylene application and are consequently believed to be due to ethylene production (Abeles 1973). Parenchyma cells of galls increase in width rather than length which is the reverse of normal development (Atkinson 1892 in Christie 1936, Dropkin and Nelson 1960, Owens and Specht 1964, Akitt 1975). Such lateral expansion of cells has been attributed to ethylene action (Burg and Burg 1966). Development of adventitious roots, a growth response attributed to ethylene action (Abeles 1973) has also been observed subsequent to Meloidogyne infestations (Orion and Minz 1969). This evidence though circumstantial indicates that ethylene biosynthesis may be stimulated in root-knot nematode infested plants.

Orion and Minz (1969) investigated the effect of 2-chloroethane phosphonic acid (ethrel) on the pathogenicity of Meloidogyne javanica infected tomato plants. This compound is readily hydrolyzed by plants to ethylene. Adventitious roots were formed and stem heights lessened in response to ethrel application to healthy plants or root-knot nematode infestation. After soil drench applications of ethrel to M. javanica infested plants, an increase in gall size to twice that of untreated infested plants was found. Histological examination revealed that the galls were not structurally altered and it was concluded that the increased size observed was a result of an increase in the proliferation of parenchyma cells. The authors hypothesized that ethylene influences the hyperplastic response and may be directly involved in the process of gall formation. Since this proposal was made, no information on ethylene contents of galled versus non-galled root tissues has been published. This study reports

directly the ethylene contents of infested and non-infested root systems.

Resistance of plants to certain bacterial and fungal pathogens has been linked to ethylene production (Sequira 1973). Stahmann et al. (1966) induced resistance in potato root tissue to black rot fungus (Ceratocystis fimbriata) by exposing the tissue to low concentrations (8 ppm) of ethylene. Clare and co-workers (1966) suspended normally susceptible potato root slices above root slices infested with C. fimbriata in a sealed container. After a 2-day incubation the uninfested susceptible slices were removed and subjected to the fungus but were found to be resistant. They concluded that the agent which conferred resistance must have been a volatile substance and further characterization identified the agent as ethylene. Thus evidence also exists that ethylene may be involved in resistant mechanisms of plants to certain pathogenic fungi. There is no information in the literature on the endogenous ethylene levels of root-knot nematode resistant and susceptible plants. Neither has there been published data on ethylene levels in resistent plants before and after exposure to root-knot nematode. It was therefore decided to investigate these proposals and establish whether ethylene is involved in plant resistance to root-knot nematodes. The host-parasite couple of Meloidogyne incognita and tomato plants was chosen. Detailed descriptions of the histopathology for this host-parasite pair are published and considerable biochemical information has been obtained with this particular combination of host and parasite. Orion and Minz (1969) used Meloidogyne javanica and tomato plants in their investigations on the influence of ethrel on root-galling.

2. LITERATURE REVIEW

LIFE HISTORY OF *Meloidogyne*

More than 30 species of the genus *Meloidogyne* have been identified (Franklin 1971). The genus is placed in the family Heteroderidae, superfamily Tylenchoidea, order Tylenchida, class Secernentea of the phylum Nematoda. Eggs of *Meloidogyne* are elongate ovate bodies, twice as long as wide. They are laid in a gelatinous matrix, the egg sac, that protrudes from the posterior of the female usually at the surface of the root gall, but sometimes within it (Christie 1936). Numbers in the range of 200-500 eggs per female are normally laid (Thorne 1971). Larvae develop within the egg assuming a long slender shape. The first larval moult occurs within the egg and infectious second stage larvae hatch. These make their way through the soil to a suitable location for penetration of a root. Penetration can occur at any point along the root but usually occurs just posterior to the apical meristem of a root tip (Christie 1936, Bird 1961). Penetration is effected by continual jabbing of the root cell wall with the nematode's stylet until a port for entry is opened. *Meloidogyne* larvae then make their way either to the provascular tissue of the elongation zone or vascular tissue in differentiated root tissues and become sessile. Infection counts in other tissues have been described for *Meloidogyne*, but are uncommon (Christie 1936). Shortly after assuming this position, cells begin to modify in response to the parasite and form characteristic giant cells (syncytia) around the head of the nematode. These are usually 4 to 7 in number and provide a source of food for the nematode. Once they are established, the parasites grow rapidly, undergoing three moults while increasing in girth but not length. After the third moult, sexes are distinguishable. Males elongate, become coiled within their third cuticle

undergo a fourth moult, and then migrate out of the root to become free living in the soil. Females enlarge in girth, becoming dorso-ventrally flattened ovoid bodies embedded in a mass of gall tissue. The egg sac develops posteriorly and usually ruptures the plant cortex tissue to lie at the surface of the gall (Thorne 1961).

Mechanism of Nematode Penetration and Infestation:

Infectious Meloidogyne larvae locate host roots by a combination of random movement and host attraction via root exudates (Endo 1975). Nematodes are equipped with a neuro-sensory system and are thus influenced by their environment. Klinger (1965) concluded that root exudates can attract nematodes from distances of 1-2 cm identifying CO_2 and certain amino acids as the attracting agents. Non-host plants (Endo 1975) and some resistant plants (Giebel 1974) lack attractive stimuli, their root exudates having no effect on the nematodes concerned. Most commonly nematodes are attracted to growing root tips where penetration is effected just posterior to the root cap (Thorne 1961, Bird 1961, Endo 1971, Christie 1936). Once an opening for penetration has been made, a number of larvae may enter through it (Thorne 1961. Bird 1961). Larvae migrate inter- or intracellularly, usually coming to rest with their heads in the central cylinder of vascular tissue (Christie 1936, Dropkin and Nelson 1960, Akitt 1975). Cells on which the nematode first feeds by inserting its stylet and removing some of the contents soon modify to become syncytia (giant cells) (Endo 1975). Bird (1967) determined that pre-parasitic Meloidogyne javanica larvae had active sub-ventral salivary glands and an inactive dorsal gland while parasitic larvae activated the dorsal gland and the sub-ventral glands become inactive.

Host Responses to Root-knot Nematodes

Morphological Modification

The host's morphological response to Meloidogyne infestation is complex. Major responses are twofold involving the production of giant cells or syncytia and the formation of galls. While the hyperplasia of tissues leading to gall formation is not essential for survival of the nematode, successful induction of syncytia are (Bird 1974).

Formation of Galls

Galls originate from hyperplasia of parenchyma cells of the cortex, pericycle and endodermis (Christie 1936, Dropkin and Nelson 1960, Bird 1961, Owens and Specht 1964, Littrell 1966, Huang 1966, Paulson and Webster 1970). Cells around the parasite apparently retain their juvenile nature when infection is in the provascular tissue, or differentiated tissues are induced to a juvenile condition which enables mitosis and gall formation to proceed (Christie 1936, Seinhorst 1961, Farooq 1973).

The first indications of galling is a hypertrophy of surrounding cells and a mitotic flare leading to gall formation follows. The time lapse required for these responses is somewhat variable but hypertrophy of vascular and corticular cells has been recorded 24 hours post-infestation (Christie 1936). It is believed that unique stimuli prompt the host responses of syncytia and gall formation. The former is thought to be a result of nematode feeding while the latter probably originates from physical pressure exerted on surrounding cells by the growing nematode or by excreted chemicals (Owens and Specht 1964, Bird 1967). Cortex parenchyma cells of galls remain mononucleate and resemble normal cells in all characteristics but their size and shape (Owens and Specht 1964, Seinhorst 1961).

Development of abnormal xylem vessels within galls has been reported (Krusberg and Nielson 1958, Paulson and Webster 1970, Farooq 1973, Jatala and Jenson 1976).

Formation of Syncytia (Giant Cells)

One of the earliest host responses to Meloidogyne infection is a hypertrophyof cells immediately surrounding the head of the parasite. This is generally observed in vascular cells but has been observed in cortical cells (Christie 1936, Bird 1974, Akitt 1975). Dedifferentiation of the cytoplasm, a condition termed anaplasia, occurs immediately after the nematode first feeds on a cell and this is followed by marked hypertrophy of the nucleus (Owens and Specht 1964). Prior to the adult moult, mature giant cells (syncytia) are well established (Owens and Specht 1964). These are characterized by their large size, many and highly labulated nuclei, dense granular cytoplasm, secondary cell wall thickenings and a proliferation of cytoplasmic organelles (Webster 1975, Endo 1975). Controversy exists as to the origin of the multinucleate condition and large size of syncytia. Early work had established cell wall breakdown of adjoining cells and a concomitant coalescing of their contents gave rise to the large multinucleate syncytia (Christie 1936, Dropkin and Nelson 1960, Bird 1961, Owens and Specht 1964, Littrell 1966). Huang and Maggenti (1969a) however, after a detailed electron microscope study reported no evidence of cell wall dissolution in M. javanica induced syncytia of Vicia faba. In contrast they reported structures generally associated with cell wall thickening which they termed boundary formations. In a further study, Huang and Maggenti (1969b) determined that syncytia underwent synchronous mitosis resulting in chromosome numbers of 4, 8, 16, 32 and 64 n. They concluded

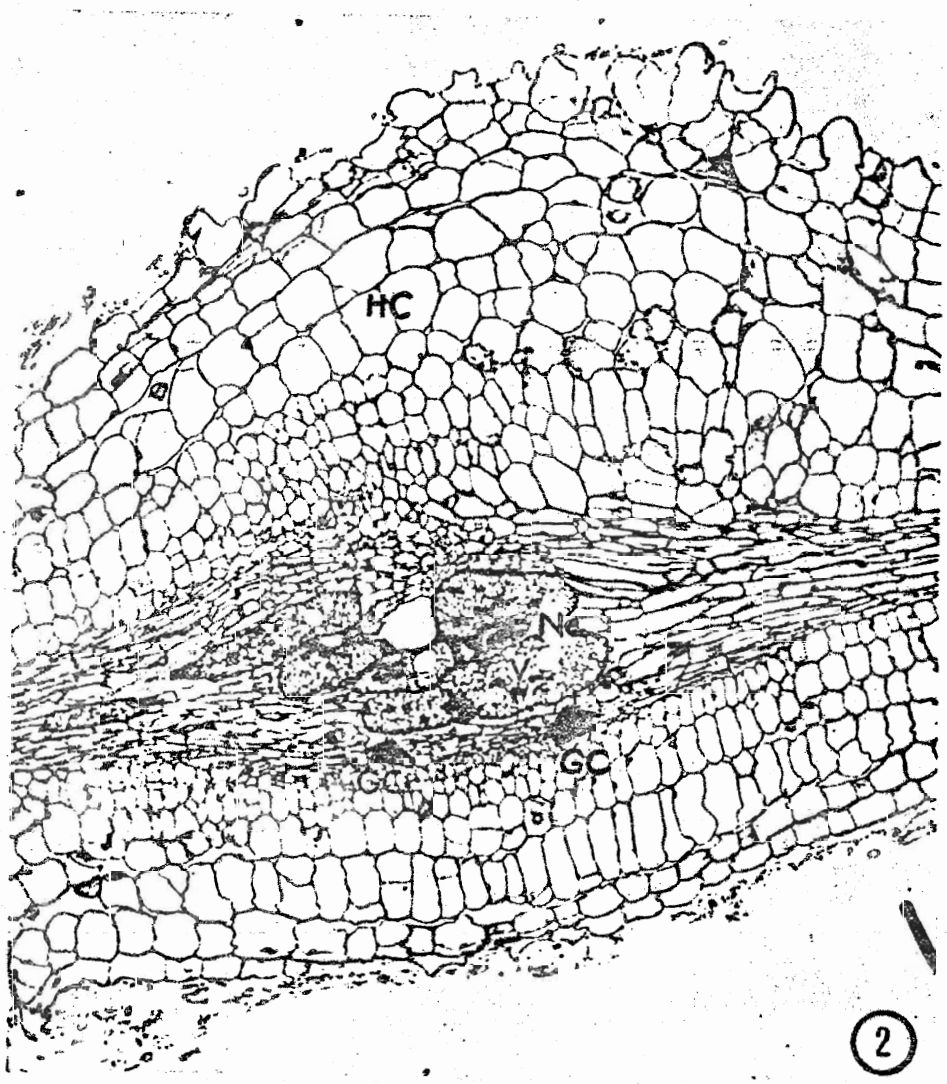
that syncytia are formed from a single cell by repeated synchronous mitosis without cytokinesis. Support for this hypothesis came from the investigations made by Paulson and Webster (1970) and Jones and Northcote (1972) who found no evidence of cell wall dissolution while also observing boundary formations. Bird (1972) made quantitative measurements of chromosome numbers in giant cells of tomato and bean plants and found numbers not consistent with the ploidy suggested by Huang and Maggenti. However, synchronous mitosis was reconfirmed in this work. Further, Bird (1973) observing 2 µm serial sections of bean syncytia found evidence of both cell wall breakdown and coalescing of cytoplasm. Recently Jatala and Jensen (1976) detected cell wall dissolution in the formation of Meloidogyne induced syncytia of Beta vulgaris. It is currently suggested that a combination of cell wall dissolution and coalescing of cytoplasm and nuclei as well as synchronous mitosis with the occasional fusion of nuclei or incomplete separation of chromosomes during mitosis gives rise to the multi-nucleated syncytia (Webster 1975, Bird 1974).

BIOCHEMICAL MODIFICATIONS INDUCED BY NEMATODE PARASITISM

DNA synthesis in developing syncytia reaches a peak just prior to egg laying (Bird 1972). RNA synthesis has been observed to increase concomitantly (Bird 1961, Rubenstein and Owens 1964).

High concentrations of protein and amino acids have been detected in a variety of host syncytia (Krusberg 1963, Setty and Wheeler 1968, Giebel and Strobeicka 1974). Intense enzymic activity has been found in syncytia especially in the early stages of their development and in the vicinity of the nematode's stylet (Endo and Veech 1969, Veech and Endo 1969a, b). Malate, succinate, isocitrate and glucose-6-phosphate oxidoreductases; 6-phospho gluconate dehydrogenase and NAD and NADP diaphorase activities were increased. As

Figure 1. Longitudinal section through a young gall. The nematode (Ne) is located in the vascular tissue where it is surrounded by syncytia (GC). Note the multinucleate condition and dense granular cytoplasm of these cells. Hypertrophied cortex cells (HC) are seen and proliferation of parenchyma cells leading to gall formation has begun. (from Paulson and Webster 1970)


HC
NC
GC
GL
2

well, oxido reductase activity was increased in cells around the anal region of the nematode and the authors postulated a connection between excreted oxido reductases and the hyperplastic galling response of the host. Increased activities of alkaline phosphatase, acid phosphatase, esterase, peroxidase, adenosine triphosphatase and cytochrome oxidase were detected in syncytia only. Orion and Bronner (1973) found increased levels of invertase and amylase in giant cells and galls of tomato roots infected with M. javanica. They postulated these were involved in the breakdown of starch to small sugars to feed the nematode or be used by the syncytia. It is generally accepted that these enzymes originate from the host in response to the parasite. However, Hussey and Sasser (1973) isolated a peroxidase in buccal exudates of M. incognita. Peroxidases are thought to be involved in the formation of lignin by plants, can act as catalysts in oxidation of proteins and amino acids and may be involved in the aerobic oxidation of indole acetic acid (IAA). All of these reactions may be involved in the formation of syncytia.

Cellulase has been found in homogenates of entire nematodes (Reidel and Mai 1971, Dropkin 1963), but it is not clear whether this enzyme is involved in syncytia cell wall breakdown since this usually occurs at the end cell walls distal to the parasite (Endo 1975). The bulk of eosophageal gland exudates are reported to be histone-like proteins (Bird 1969), an interesting result considering the role of histones in gene regulation.

Thus root-knot nematode induced syncytia are highly specialized cells induced to develop from unspecialized cells by the parasite. That the initial trigger for their development comes from feeding and subsequent deposition of saliva within these cells is probable. It has been established

that mature syncytia are essential for the development and survival of the parasite and removal of the parasite results in degeneration of these structures (Bird 1962). Thus a perpetual stimulus is needed for their continuation. Syncytia have been termed transfer cells specialized for selective transfer of metabolites over a short distance, in this case providing food for the parasite (Bird 1975). Many host cell modifications in the formation of syncytia may involve changes in gene expression, which may be regulated by nematode secreted histones or plant hormones.

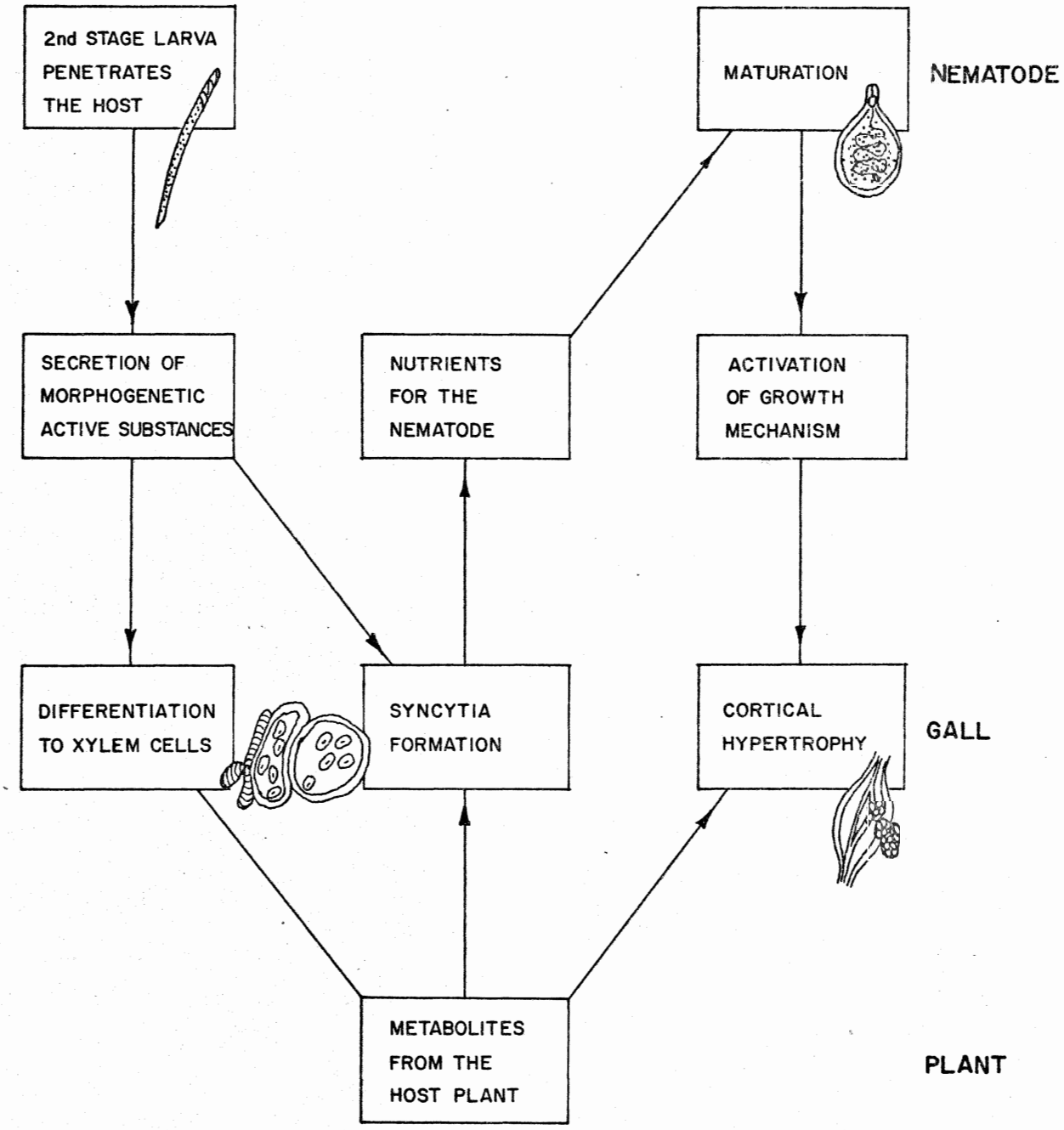
ROLE OF PLANT GROWTH HORMONES IN HOST RESPONSES

Normal plant growth is regulated by the action of plant hormones. The likelihood that modifications in normal patterns of growth as a result of root-knot nematode infection are mediated by hormones has received much attention. Many of the changes that occur in the development of syncytia and galls mimic hormone action in healthy plants. Hypertrophy of cells in the vicinity of the infection site and their subsequent elongation and growth suggests the involvement of growth promoting auxins and gibberellins. Intense mitotic activity of cortex parenchyma resulting in galls may well involve cytokinins which are known to stimulate plant cell division. Auxins and gibberellins are known to have stimulatory effects on the synthesis and activities of enzymes. It has long been speculated that nematode interactions with endogenous plant hormones may trigger these changes in growth.

ROLE OF AUXINS IN THE SUSCEPTIBLE RESPONSE

Auxins are found in highest concentrations in meristematic tissues. They are often conjugated with other compounds (sugars, polysaccharides,

Figure 2. Essential steps in stimulation of gall formation by nematodes. Each arrow represents an essential process for a successful host-parasite relationship. (from Orion 1973).


2nd STAGE LARVA PENETRATES THE HOST
MATURATION
NEMATODE
SECRETION OF MORPHOGENETIC ACTIVE SUBSTANCES
NUTRIENTS FOR THE NEMATODE
ACTIVATION OF GROWTH MECHANISM
DIFFERENTIATION TO XYLEM CELLS
SYNCYTIA FORMATION
CORTICAL HYPERTROPHY
GALL
METABOLITES FROM THE HOST PLANT
PLANT

proteins and amino acids) in a physiologically inactive form (Bidwell 1974). Yu and Viglierchio (1964) determined endogenous levels of auxins in galls of tomato roots infested with three species of root-knot nematode, Meloidogyne hapla M. incognita and M. javanica. They also determined auxin distributions in homogenates of the parasites themselves. Generally the number and type of auxin found in the galls correlated with the number and type found in the nematodes responsible for the galls. M. hapla homogenates and gall preparations both contained indole acetic acid (IAA), indole acetic acid ethyl ester (IAE) and indoacetonitrile (IAN). M. javanica homogenates and galls contained IAA and IAN while those of M. incognita contained only indole butyric acid (IBA). Since only one variety of host plant was used (Pearson) they concluded that the nematode was responsible for the number and type of auxins produced in the affected tissues. Extrapolating this line of reasoning it could be postulated that the nematodes secreted auxins much the same as the gall-inducing insects do (Miles and Lloyd 1967, Miles 1968). However, the suggestion that they were ingested subsequent to infestation (Mountain 1960a) cannot be discounted. Sandstedt and Schuster (1966) determined that M. incognita did not secrete cytokinins or auxins in tissue culture preparations and M. hapla exudates were also found to be devoid of auxins, gibberellins or cytokinins (Bird 1966). It is now well established that greater auxin concentrations are found in galls than uninfected root tissues (Balasubramanian and Rangaswami 1962, Bird 1962, Viglierchio and Yu 1965, Brueske and Bergeson 1972).

The question arises as to the source of these auxins. The first possibility is that nematodes secrete proteases and glycosidases (or in some way trigger their activity) and these release free auxins from conjugates

with amino acids or sugars. Further, the hydrolysis of proteins would supply the necessary tryptophan for subsequent increased synthesis of auxins (Giebel 1974). Conversely it has been suggested that auxins originate in the nematodes as detoxification products and that these are subsequently excreted into the host by the parasite (Viglierchio 1971). This would account for the correlation of number and types of auxins found in nematodes and their respective galls. However, auxin effects would be expected in cells around the anal region and syncytia are formed only around the nematodes head in cells on which it has fed. This would not however preclude a possible role of auxins in the hyperplastic galling response. Transfer of auxins from nematode to host has not been observed (Viglierchio 1971).

Amino acids occur in greater concentrations in galls than healthy host root tissue (Owens and Specht 1966, Owens and Rubenstein 1966). These may be of nematode origin (Lee 1965) or may arise through the release and/or activation of proteolytic enzymes (Endo and Veech 1969). Favoured pathways of IAA synthesis in plants convert tryptophan via tryptamine, indole pyruvic acid or indole acetonitrile to IAA. Presumably auxin synthesis would increase as a response to the increase of available substrate as has been observed for microorganisms (Matsuyama and Misawi 1963).

Orion (1973) found no influence on root-knot nematode development or host gall structure with exogenous application of alar B-9 an auxin inhibitor. Exogenous application of the auxin naphthalene acetic acid (NAA) did not aid nematode development in tissue cultures where they developed slowly or not at all. NAA applications to susceptible tomatoes infested with M. incognita had no effect on the degree of infestation or host response (Sahney and Webster 1975). Applications of NAA and cytokinins together to root-knot nematode infested plants or tissue cultures resulted in increased severity

of infection (Dropkin et al. 1969, Kochba and Samish 1971) or aided the development of nematodes to maturity through the necessary initiation of syncytia in tissue cultures (Sandstedt and Schuster 1966). Omission of one or the other hormone resulted in no syncytia formation and the nematodes failed to develop.

Tobacco pith cells in tissue cultures were observed to undergo cell expansion and mitosis without cytokinesis, resulting in large multinucleated cells when IAA was supplied to the medium and kinetin withheld. Addition of kinetin prompted cytokinesis and normal cell divisions (Naylor et al. 1954). In the natural condition plants apparently need both hormones for normal cell divisions. The response of cultured tobacco cells to IAA in the absence of kinetin is similar to that observed in the formation of nematode induced syncytia and it is tempting to speculate that a similar mechanism may be involved.

BIOCHEMICAL AND HORMONAL MODIFICATIONS ASSOCIATED WITH RESISTANCE.

A full understanding of hormonal and biochemical actions mediating the susceptible response can only be obtained by examining these relationships in the resistant response. Mechanisms of resistance to root-knot nematode infection can be grouped into four categories:

(1) The plant produces toxic substances which kill the parasite (Uhlenbroek and Bijloo 1959),

(2) Root exudates do not attract the nematode species concerned (Jatala and Russell 1972),

(3) Substances essential to the development of the nematode within the host roots are absent or present in insufficient amounts (Webster 1969),

(4) Infested plant tissues undergo a hypersensitive response which isolates the parasitic larva in necrotic cells resulting in severely delayed nematode development or more commonly its death (Hung and Rohde 1973).

It has been reported for some plants resistant to root-knot nematodes, that the larvae are unable to initiate essential host responses and migrate out of the tissue to seek a new host (Reynolds et al. 1970). In other cases nematode development is retarded and greater numbers of males develop which leave the roots to become free-living (Fossuliotus et al. 1970). Poor syncytia development has been correlated with this shift in the sex ratio (Triantaphyllou 1973). Commonly the host undergoes a hypersensitive response (HR) to isolate the pathogen and usually kill it. Paulson and Webster (1972) examined changes in ultrastructure during the HR of resistant tomatoes (var. Nematex) to M. incognita. Necrosis of the cells immediately surrounding the parasite occurred through the breakdown of cytoplasm and cell membranes. They suggested that only cells in which the nematode had fed and deposited salivary secretions underwent the HR. It was proposed that plant lysosomes were released in response to nematode secretions 8-12 hours after penetration and these prevented the massive selective transport of solutes necessary for syncytia formation. In the absence of syncytia the nematodes starved.

Hypersensitive responses to root-knot nematodes in various other resistant hosts involved initiation of syncytia formation, however by day 4 post-infestation these began to degenerate being characterized at day 7 with extensive cell wall thickenings (Riggs et al. 1973, Chang et al. 1973, Webster 1975). Riggs et al. (1973) proposed extensive cell wall thickening and lignification sealed off plasmodesmata and selective solute transport was inhibited.

PHENOLS AND RESISTANCE

Singh and Choudhury (1973) found a direct correlation of phenol levels to Meloidogyne resistance in tomato plants where the most resistant plants had greatest amounts of total phenols and susceptible plants the least. Other researchers have substantiated these findings in a variety of plant-nematode associations (Hung and Rohde 1973, Pitcher et al. 1960, Brueske and Dropkin 1973, Giebel 1970). Plant phenols occur naturally as physiologically inactive glycosides (Giebel 1970). Meloidogyne eosophageal secretions may contain β-glucosidases capable of hydrolyzing phenol glycosides to free phenols (Bird 1969). Pratylenchus penetrans, a nematode parasitic in this case on peach roots, secreted β-glucosidases which hydrolysed amygdaline to hydrocyanic acid and benzaldehyde. These products were toxic to the parasite resulting in its death and a browning of the host tissues about the infection court occurred (Mountain and Patrick 1959).

Giebel et al. (1966) established that β-glucosidase introduced into potato roots with a micropipette caused necrosis in resistant plants and giant cells in susceptible plants. β-glucosidase and β-galactosidase activities were detected in Heterodera rostochiensis (same family as Meloidogyne) while only β-glucosidase activity was found in H. pallida. The nematodes had different pathogenicities on the same host (potato) which was directly proportional to the β-glucosidase activity of their saliva (Wilski and Giebel 1966). IAA, kinetin, phenols and aglucones were determined to be produced as a result of β-glucosidase activity (Giebel et al. 1971). These products were more abundant in necrotic cells than in syncytia. Aglucones are inhibitors or cofactors for per oxidase which can influence IAA-oxidase activity. Polyphenols have been found to

enhance the influence of IAA while monophenols are antagonistic (Tomaszewiski and Thimann 1966). Root extracts of Solanaceae which were susceptible to H. rostochiensis had low ratios of monophenols to polyphenols whereas resistant root extracts had high monophenol to polyphenol ratios (Giebel 1970). Introduction of a phenol fraction from resistant plants to susceptible plants resulted in a shift to resistance of the formerly susceptible plants. The reverse process was also observed (Wilski and Giebel 1972). The authors propose that phenols affect the activity of IAA-oxidase so that a low ratio of monophenols to polyphenols inhibited IAA-oxidase and a susceptible response occurred since IAA concentration could increase. Conversely it was proposed that a high monophenol to polyphenol ratio established resistance by increasing IAA-oxidase activity and the subsequent breakdown of IAA. Wilski and Giebel (1971) determined IAA-oxidase activity in resistant potato roots was 22% greater than in roots susceptible to H. rostochiensis. The process of cell wall thickening and IAA-stimulated growth are mutually exclusive since only cells with primary walls can grow (Siegel 1953). It was postulated that high IAA-oxidase activity associated with resistance enabled lignification to proceed (Giebel 1974).

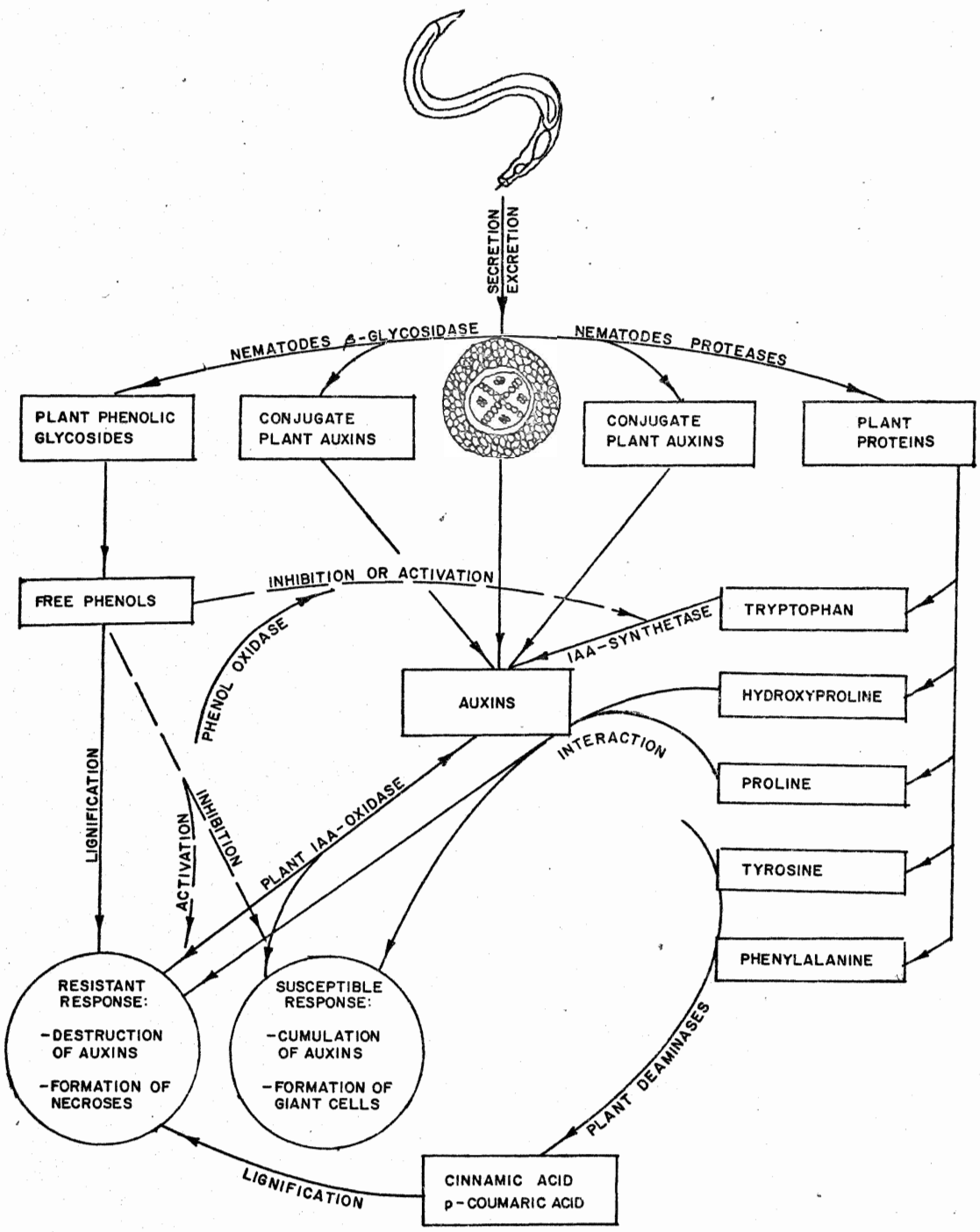
Giebel (1973) found elevated activities of the deaminases, phenylalanine ammonia-lyase and tyrosine ammonia-lyase in roots of resistant compared to susceptible potatoes. The products of these enzymes are cinnamic acid and p-coumaric acid respectively, both of which are lignin precursors and IAA-oxidase cofactors. Introduction of these acids to susceptible potato roots resulted in resistance to H. rostochiensis.

Roots of susceptible and resistant Solanaceae varieties had proline to hydroxyproline ratios of 1.59-2.11 and 2.20-2.54 respectively (Giebel and Strobiecka 1974). Subsequent Heterodera rostochiensis infestation increased this ratio to 2.30-2.36 in susceptible varieties while it was reduced to 0.93-1.23 in resistant varieties. Hydroxyproline is known to inhibit IAA-stimulated Avena coleoptile elongation and this may be reversed by proline (Cleland 1967). It may be that the relative amounts of proline and hydroxyproline interact with auxin to affect the host pathogenicity. These suggestions are shown schematically in Figure 3.

OTHER HORMONES IN THE SUSCEPTIBLE AND RESISTANT HOST RESPONSES

It was noted previously in this review that exogenous application of cytokinins and auxins together increased root-knot nematode susceptibility while applications of either separately had no effect (Sawhney and Webster 1975, Sandstedt and Schuster 1966, Dropkin et al. 1969) In contrast, resistance to M. javanica in peach roots was lessened by NAA and kinetin either applied together or individually (Kochba and Samish 1971). Dropkin and co-workers (1969) observed a reversal of resistance in tomato plants (var. Nemared) to M. incognita infestation with exogenous applications of four different cytokinins. In resistant plants prior to 0.8 μM kinetin application 40% of the nematode larvae in contact with the roots developed to maturity. Subsequent to hormone application, this increased to 57%. Necrosis of roots which coincided with death of the nematode was reduced from 88% to 31% and gall formation increased from 29% to 65% of total root weight. Exogenous application of IAA, gibberellic acid, adenine, guanine, thymine, cytidine and 6-methylamino purine had no effect.

Figure 3. Schematic representation of the hypothetical central role of auxins in plant response to root-knot nematodes (from Giebel 1974).


SECRETION
EXCRETION
NEMATODES β-GLYCOSIDASE
NEMATODES PROTEASES
PLANT PHENOLIC GLYCOSIDES
CONJUGATE PLANT AUXINS
CONJUGATE PLANT AUXINS
PLANT PROTEINS
FREE PHENOLS
INHIBITION OR ACTIVATION
TRYPTOPHAN
IAA-SYNTHETASE
PHENOL OXIDASE
AUXINS
HYDROXYPROLINE
INTERACTION
PROLINE
LIGNIFICATION
ACTIVATION
INHIBITION
PLANT IAA-OXIDASE
TYROSINE
PHENYLALANINE
RESISTANT RESPONSE:
-DESTRUCTION OF AUXINS
-FORMATION OF NECROSES
SUSCEPTIBLE RESPONSE:
-CUMULATION OF AUXINS
-FORMATION OF GIANT CELLS
PLANT DEAMINASES
LIGNIFICATION
CINNAMIC ACID
p-COUMARIC ACID

However, Sawhney and Webster (1975) repeated this experiment (i.e., M. incognita on Nemared var. tomato) and found that only NAA and kinetin when applied together reduced resistance, and that individual applications had no effect. Further they concluded that resistance was only partially broken by this treatment and assumed other factors must also be involved in the resistant mechanism. It may be that slightly different types of resistance are involved in peach and tomato since peach resistance has been shown to involve incomplete syncytia development while Nemared var. tomato undergo a HR. Kinetin lessens the browning response of resistant tissue while not affecting the HR of necrotic cells about the parasite (Webster 1974) and thus may be able to reverse resistance alone where the HR is not part of the resistant mechanism.

Peach roots resistant to M. javanica had lower levels of cytokinins and auxins than susceptible roots (Kochba and Samish 1972). Orion (1973) found exogenous applications of the anticytokinin agent, chlorfluorenal, inhibited syncytia formation and consequently drastically reduced nematode development. It appears from these results that auxins and cytokinins play an important role in determining a successful host-parasite association. Brueske and Bergeson (1972) extracted gibberellin and cytokinin activities from nematode induced gall tissues and xylem exudates. Bioassays indicated that gibberellin activities of gall tissues were decreased in three out of four different fractions. This result is inconsistent with that of Orion. Cytokinin activity of gall tissue was also decreased relative to healthy root tissues. Xylem exudates increased in gibberellic acid activities of acidic fractions while gibberellic acid activity of neutral fractions decreased subsequent to nematode infestation. Cytokinin activities of

xylem exudates of infested plants also decreased. The authors postulated that Meloidogyne incognita infestation altered the translocation process of gibberellic acids and depressed the synthesis of these and cytokinins in gall tissue.

A decrease in photosynthesis has been observed in root-knot nematode infested plants (Loveys and Bird 1973, Bird 1974). Pruning of control and infested plants to remove leaves resulted in increased photosynthetic rates of remaining leaves while little or no change was observed in leaves of infested plants (Loveys and Bird 1973). The response observed in control plants has been well documented and is believed to arise from increases of growth factors translocated from the roots (Bird 1964). Inhibition of photosynthesis in response to nematode infestation may constitute further evidence for interference of the root translocation process by the parasite.

Orion and Minz (1968) reported that (2-chloroethyl)trimethylammonium chloride (CCC), an antigibberellin agent doubled the size of galls when applied exogenously over a two month period. However, similar applications of gibberellic acid had no effect on gall size, a result found in other laboratories also (Peacock 1960). Later, Orion (1973) reported that another antigibberellin agent, phosphon D, severely inhibited development of M. javanica on tomato roots.

It should be stressed that simply applying growth promoting or inhibiting agents to plants can have a multitude of effects on overall plant metabolism. One must use caution in interpreting the results obtained by those methods. Applications of antigibberellin agents, for example, should affect plant growth by blocking all metabolic processes under gibberellic acid control. The effects on host plant metabolism would

be far reaching. An isolated aspect of plant metabolism altered by such methods cannot be attributed to blockage of gibberellic acid. The observed effect may actually be a secondary or lower order response. In addition, direct measurements of the influence of these agents on hormone levels should be made before any interpretation of results.

ETHYLENE 1. REGULATION OF PLANT GROWTH BY ETHYLENE

Ethylene has varied and sometimes opposing effects on plant development when applied to a variety of tissues. The classical 'triple response' of etiolated pea seedlings was long used as an ethylene bioassay. Seedlings when grown in dark for 7 days are long, straight and slender with a recurved apical hook. Exogenous applications of ethylene results in an inhibition of longitudinal extension while lateral expansion is observed most especially immediately posterior to the apical bud. Seedlings are also marked by a loss of their normal response to gravity (Galston and Davies 1970). The extent of these responses is directly proportional to the amount of ethylene to which the seedlings are exposed, enabling this system to be used as a bioassay. The limitations of this are many however, not the least of which is the time needed for growing the seedlings (7 days).

Root growth is inhibited, root hairs proliferate and the roots become ageotropic when ethylene is applied exogenously (Chadwick and Burg 1967, Appelbaum and Burg 1972a,b). Induction of roots from leaves and stems as well as from preexisting roots has been attributed to ethylene action (Abeles 1973). Ethylene is involved in breaking the dormancy of seeds (Toole *et al.* 1964), tubers, bulbs and buds (Abeles 1973). Etiolation, the growth response of seedlings grown in the dark, is apparently regulated by ethylene. Epinasty, a downward bending of the leaves caused by expansion

of cells on the upper surface of the petiole, is an ethylene mediated plant growth response. Other plant growth responses involving ethylene action include fruit ripening and the climacteric, inhibition of flowering, hypertrophy of the cortex and other tissues, leaf senescence and abscission. As well, ethylene production increases in plants subsequent to stress (i.e., mechanical damage) and has been implicated in promoting disease resistance of some plants to certain pathogens (see introduction, Sequira 1973, Abeles 1973).

Inhibition of growth by ethylene has been attributed to a retardation of mitosis in meristematic tissue of roots, shoots and buds (Apelbaum and Burg 1972a,b). Auxin applied exogenously also retards growth in a similar fashion and it is now established that this is at least partly due to auxin induced ethylene productivities (Webster and Davidson 1967). The inhibitory effects of ethylene and auxin (probably through auxin stimulated ethylene synthesis) on the mitotic process of meristematic tissues can be reversed by the application of cytokinin (Burg and Burg 1967, 1968, Wickson and Thimann 1958). It is enticing to predict that higher plants control apical dominance through the correlative amounts of these three hormones in the buds but this has not been unequivocally proven. The issue is confused in that ethylene while known to inhibit mitosis in meristematic tissue was also observed to break dormancy and apical dominance (Kang and Burg 1972). Increased ethylene production in non-dormant versus dormant seeds and an increase in ethylene evolved just prior to germination have been observed (Esashi and Leopold 1970).

Although stem elongation of dicotyledons is inhibited by ethylene, monocotyledons, have shown ethylene stimulated growth (Abeles 1973).

Imaseki and Pjon (1970) determined that ethylene did not affect growth of rice coleoptiles alone but had an enhancing effect on auxin stimulated growth. Such effects of ethylene on auxin action have been reported in the induction of adventitious roots as well (Krishnamoorthy 1970).

Lateral swelling of cells to ethylene is well documented (Abeles 1973). Associated with this swelling in etiolated pea seedlings is a retardation of differentiation (Apelbaum and Burg 1972a). In addition, lignification processes are concurrently inhibited (Apelbaum et al. 1972). These actions produce hypertrophied cells and it is tempting to associate this action of ethylene to the hypertrophy of cells encountered shortly after root-knot nematode penetration of host tissues. However, there is no experimental confirmation of this suggestion.

Cellulose microfibrils are normally deposited transversely in the cell wall, an orientation which limits lateral expansion and favours longitudinal extension. Ethylene disrupts this condition so that the cellulose microfibrils are laid down longitudinally and lateral expansion is preferred while extension is limited (Eisinger and Burg 1972). Excess auxin concentrations result in the same phenomenon as a result of auxin induced ethylene synthesis (Chadwick and Burg 1970).

Epinasty is thought to be a result of auxin-induced cell overgrowth of the upper basal cells of the petiole causing the leaf to bend downward (Palmer 1972). Lyon (1970) determined that ethylene inhibited gravity dependent lateral transport of auxin in petioles. Clinostat studies determined that in the presence of ethylene excess auxin was delivered to the upper side of leaves. It was proposed that ethylene blocked gravity dependent transport of auxin to the lower side of the leaf. Similarly ethylene inhibited geotropic curving of roots (Chadwick and Burg 1966)

and phototropism of stems of mustard, radish and pea seedlings (Burg and Burg 1966). Burg and Burg (1966) investigated ethylene effects on the transport of [^{14}C]indoleacetic acid in pea seedlings and corn coleoptiles. It was established that the lateral auxin transport system was essential for the normal geotropic responses of pea seedlings laid on their sides (i.e., stems curve upwards and roots down) and that ethylene inhibited these responses. The evidence strongly supports the theory that ethylene interferes with the lateral auxin transport system resulting in a loss of tropic sensitivity to gravity and light.

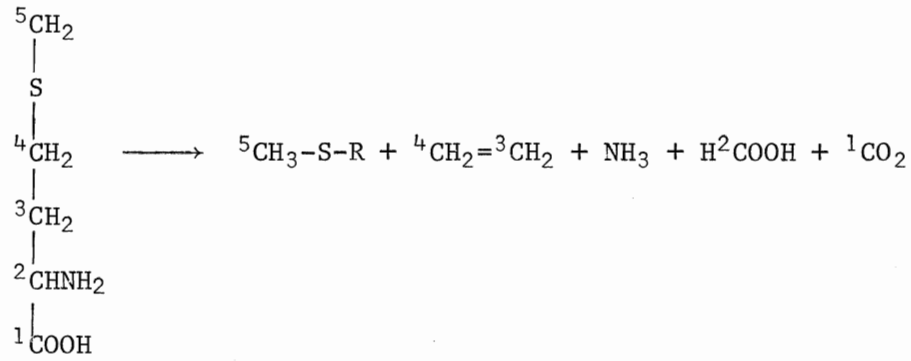
2. MECHANISM OF ETHYLENE ACTION

Analogue studies have revealed that molecules with ethylene action have a terminal carbon adjacent to a double bond (Burg and Burg 1967). Biological activity is directly proportional to the ability of analogues to bind metals and it was suggested that ethylene binds to a receptor molecule at a metal containing active site with a $K_m = 6 \times 10^{-10}$ M (Burg and Burg 1967). The bond is non-covalent since no exchange of deuterium takes place when deuterated ethylene is used (Beyer 1972). This result was substantiated by the observation that most ethylene induced responses are readily reversible (Galston and Davies 1970). Carbon dioxide is a competitive inhibitor of ethylene action (Chadwick and Burg 1967).

3. BIOSYNTHESIS OF ETHYLENE

It is generally accepted that methionine is the in vivo precursor of ethylene (Abeles 1973). Burg and Clagett (1967) followed the conversion of ^{14}C-methionine to ethylene. They determined carbons 3 and 4 were released as ethylene, carbon 1 as CO_2, carbon 2 as formate and the S-methyl was

widely distributed and metabolized



The process is aerobic with a wide range of pO_2 reported, however, these discrepancies have been explained as due to liquid phase shifts encountered when tissues are incubated in liquid medium. Dry apple discs flushed with N_2 immediately stopped ethylene production while those floated on buffer solutions produced ethylene for an hour after N_2 had been bubbled through the solution (Imaseki and Burg 1972). The K_m was 20% O_2 for tissue treated this way but by omitting the liquid phase (i.e., apple discs in air), a K_m of 0.2% O_2 for ethylene production was found. This high affinity for O_2 indicates that cytochrome oxidase and the respiratory electron transport system was involved and not an oxidase specific for ethylene synthesis. The decarboxylation is an oxidative process closely related to the evolution of ethylene as determined by inhibitor studies (Baur et al. 1971). That respiratory poisons inhibit in vivo ethylene production lead Burg (1973) to propose the existence of a high energy intermediate. Further it was proposed that this might be S-adenosyl methionine as large quantities of it were obtained from ^{14}C-methionine fed apple discs as were the known products of ethylene synthesis from methionine (Burg and Clagett 1967).

The site for ethylene synthesis in the cell appears to be either the mitochondria (Meheriuk and Spencer 1964, 1967a,b) or the chloroplast

(Elstner *et al.* 1976). *In vitro* model systems producing ethylene from methionine and especially its derivative methional in the presence of Cu^{+} and peroxide have been reported (Lieberman *et al.* 1966). The methionine analogue 2-keto-4-methylthiobutyrate (KMBA) can be degraded *in vitro* by peroxidase in the presence of Mn^{2+} or peroxide, pyridoxal-phosphate and a monophenol. Ethylene is formed from carbons 1 and 2 and the S-methyl portion is turned into dimethyl mercaptan (Ku *et al.* 1969). A transaminase which converts methionine to its 2-keto derivative KMBA has been observed (Durham *et al.* 1972). A problem arises in that the *in vitro* system produced ethylene from carbons 1 and 2 while the reported *in vivo* production of ethylene is from carbons 3 and 4 of methionine (Burg and Clagett 1967). However, if peroxidase is the enzyme involved in ethylene biosynthesis the autocatalytic activity of ethylene synthesis may be explained in that peroxidase enzyme production is induced by ethylene action (Imaseki 1970). The production of phenols in response to ethylene is well documented (Sequira 1973) and these are involved in the mechanisms of disease resistance, fruit ripening and senescence (Abeles 1973). Some isozymes of peroxidase produce ethylene more readily than others and since auxin stimulates production of some of these isoperoxidases it has been suggested that this may be the mechanism by which auxin stimulates ethylene production (Galston and Davies 1970).

A model system has been developed by Elstner *et al.* (1976) whereby chlorophyll free extracts from sugar beet leaves were stimulated to produce superoxide free radical ion from 3-hydroxytyrosine in illuminated chloroplast lamellae. The superoxide free radical ion was determined to be a

prerequisite for ethylene formation from methional and was dependent upon a lamellar phenol oxidase and a photosynthetic electron transport 1 electron donor. It was suggested that this system may be the *in vivo* ethylene synthetic mechanism induced by wounding of plant tissues.

3. MATERIALS

3-1. Biological Materials

Seeds of tomato, Lycopersicon esculentum variety Vendor, Tiny Tim, and Glamour, were purchased from Stokes Seeds Limited, St. Catharines, Ontario. Tomato seeds of variety Anahu were supplied by the Horticulture Experiment Station of the Ontario Ministry of Agriculture and Food, Simcoe, Ontario. Root-knot nematodes, Meloidogyne incognita were obtained from the Canada Department of Agriculture Research Station, Vineland Station, Ontario.

3-2. Chemicals

All commercial chemicals were purchased from BDH (Canada) Ltd. or Fisher Scientific Company, New Jersey, U.S.A. and were of analytical grade. The following are notable exceptions and specialty products:

(a) ethane (C_2H_6) C.P. Grade Union Carbide Canada Limited, Toronto

(b) ethylene (C_2H_4) C.P. Grade Union Carbide Canada Limited, Toronto

(c) hydrogen (H_2) prepurified Union Carbide Canada Limited, Toronto

(d) nitrogen (N_2) prepurified Union Carbide Canada Limited, Toronto

(e) Indicarb 10-20 mesh (CO_2 absorber)
Fisher Scientific Co., New Jersey, U.S.A.

(f) Molecular Sieves Type 4Å (1.6 mm pellets)
Fisher Scientific Co., New Jersey, U.S.A.

(g) Silica Gel (100 mesh) Fisher Scientific Co., New Jersey, U.S.A.

(h) Polyoxyethylene sorbitan monolaurate (Tween 20)
Sigma Chemical Co., St. Louis, Mo., U.S.A.

(i) Rubber 'O' rings (0.64 cm diameter)
Chromatographic Specialities Ltd., Brockville, Ontario.

(j) Rubber septa (0.64 cm diameter)

Chromatographic Specialities Ltd.,

Brockville, Ontario.

(k) Stopcock grease-silicone lubricant

Dow-Corning Company Limited,

Midland, Michigan, U. S. A.

(l) Vermiculite (industrial number 4 grade--fine)

Ball Superior Ltd., Mississauga, Ontario

(m) No-Damp Fungicide (oxine benzoate 2.5%)

Plant Products Co. Ltd., Bramalea, Ontario

(n) Greenhouse Dibrom Insecticide (nalid 36%)

Chevron Chemical Canada Ltd.,

Ortho Division, Burlington, Ontario

(o) Lindane 25 W.P. Insecticide Chevron Chemical Canada Ltd.,

Ortho Division, Burlington, Ontario

(p) Malathione 50 Insecticide Chevron Chemical Canada Ltd.,

Ortho Division, Burlington, Ontario

(q) Meta-Systox R (oxydeneton-methyl 25%)

Chevron Chemical Canada Ltd.,

Ortho Division, Burlington, Ontario

3-3. Reagents

Mercuric perchlorate solution

40 ml distilled water and 210 ml 60% perchloric acid were added to a large glass mortar. 54.2 grams of red mercuric oxide were added slowly while grinding with the pestle to prevent caking. The solution was filtered through a sintered glass funnel, diluted to 1 liter with distilled water and stored in a glass reagent bottle. Mercuric perchlorate has a

shelf life (at room temperature) of about one year. All surfaces on which some of this solution may have been spilled were flushed thoroughly with water due to the explosive properties of mercuric perchlorate.

4N Lithium chloride

42.4 grams of lithium chloride were dissolved in about 200 ml of distilled water and diluted to 250 ml in a volumetric flask.

Ammonium sulphate solution

71 grams of ammonium sulphate $(NH_4)_2SO_4$ were dissolved in every 100 ml of distilled water. After this had dissolved, an additional 10 grams per 100 ml of solution were added to maintain a slight excess of salt and keep the solution saturated.

0.01% Polyoxyethylene sorbitan monolaurate (Tween 20)

1.0 ml Tween 20 was diluted up to 100 ml with distilled water to prepare a stock solution of 1.0% Tween 20. 10 ml of stock solution were diluted to 1 liter with distilled water to provide 0.01% Tween 20 working solution.

Lactophenol solution[1]

Solid phenol (acid carbolic) was melted on a water bath to provide 500 ml of liquid. To this were added 500 ml lactic acid, 500 ml distilled water and 1000 ml of glycerol. The solution was allowed to stand at room temperature.

---

[1] Extreme caution should be observed in the preparation and use of this solution due to the presence of phenol and phenol fumes.

Cotton Blue-Lactophenol

To obtain a 0.1% solution of stain, 1.0 gram aniline blue, water soluble, was added to 1000 ml lactophenol solution.

Stock Cotton Blue-Lactophenol was also purchased in 100 ml quantities from British Drug Houses at a concentration of 0.125%. Appropriate dilutions of this stock solution were also used to prepare the desired working concentration of stain.

Destain Solution

1 gram of zinc chloride was dissolved in every 1.7 ml of 12 N HCl used up to the desired volume.

Table 1. Hydroponics Solution: Schedule for 21 litres of 7x Strength Stock Solution

Salt	Molecular Weight	Concentration of Stock Solution	grams/litre of Salt for Stock Solution	ml/litre of Stock for Working Solution	ml/21 litres of Stock for 7x Concentrate
Potassium Nitrate, KNO_3	101.10	1 M	101	6	882
Calcium Nitrate, $Cu(NO_3)_2$	236.16	1 M	236	4	588
Ammonium Phosphate, $NH_4H_2PO_4$	115.08	1 M	115	2	294
Magnesium Sulphate, $MgSO_4$	246.49	1 M	246	1	147
Micronutrients[1]					
Potassium Chloride, KCl	74.55	50 mM	3,728	1	147
Boric Acid, H_3BO_3	61.84	25 mM	1.546	1	147
Manganese Sulphate, $MnSO_4$	169.01	5 mM	0.845	1	147
Zinc Sulphate, $ZnSO_4$	287.55	2 mM	0.575	1	147
Copper Sulphate, $CuSO_4 \cdot 5H_2O$	249.71	0.5 mM	0.125	1	147
Molybdic Acid, H_2MoO_4	161.97	0.1 µM	0.017	1	147
Iron[2]					
Ferrous Sulphate, $FeSO_4$	278.03	2 µM	0.556	2	294

[1] Micronutrient salts are combined to make one stock solution

[2] Iron stock solution was adjusted to pH 3.5 with sulphuric acid

from Rickels (1973)

4. METHODS

4-1. Experimental Design and Logistics

This section is intended to describe the overall experimental protocol and approach, details of individual procedures follow in later sections. Six complete experiments were conducted during the course of this research, four involving susceptible plants and two involving resistant plants. Experiments were begun by infecting tomato root systems with nematodes (Meloidogyne incognita) and data were gathered over the subsequent thirty day periods. Under most conditions this species of nematode has a life cycle of less than 30 days. Experimental data was normally collected every other day with a series of procedures which are termed an experimental run. Most experiments then consisted of fourteen to sixteen experimental runs over a thirty day period. Each run utilized four plant samples; two control and two infested. Each sample consisted of one plant except in Experiment 1 (four plants per sample) and Experiment 2 (two plants per sample). Thus, Experiment 1 consumed 256 plants, Experiment 2, 128 plants and the rest of the experiments sixty plants. For each sample the stem height, top weight and root weight were measured. In addition the root gases were extracted and analyzed.

4-2. Germination and Growth of Tomato Plants

Seeds of tomato variety Vendor (susceptible) and Anahu (resistant) were soaked in distilled water with gentle stirring for twenty-four hours. The seeds were arranged on tissue laid of top of vermiculite-water 1-1.5 (v-v), the container covered with a glass plate to maintain high humidity. After four days of incubation in the dark at room temperature, seedlings

were removed to the greenhouse and uncovered. After a further seven to ten days when approximately 5 cm high, the plants were transplanted into sterile sand in 10 cm x 10 cm square pots (Experiment 1 in 5 cm x 5 cm square pots). These were arranged on benches under a bank of fluorescent lights (General Electric Reflector cool white FR96T12. CW-1500) and exposed to a sixteen hour photoperiod.

Seedlings were treated with oxine benzoate, a fungicide, to prevent Damping Off disease. Growing plants were watered with a balanced nutrient hydroponics solution (Rickels, 1973) diluted to half-strength. White fly (Trialeurodes vaporariorum) populations, a pest of the growing plants, were kept in check with various spray insecticides. Plants of Experiment 1 were infected with nematodes when forty-six days old. All other experimental plants were infected after ninety to 125 days after germination depending on their rate of growth. At this point, the plants had an extensive root system and had started to flower. Plants were maintained in the greenhouse until they were used in the experimental runs.

4-3. Propagation and Harvesting of Nematodes

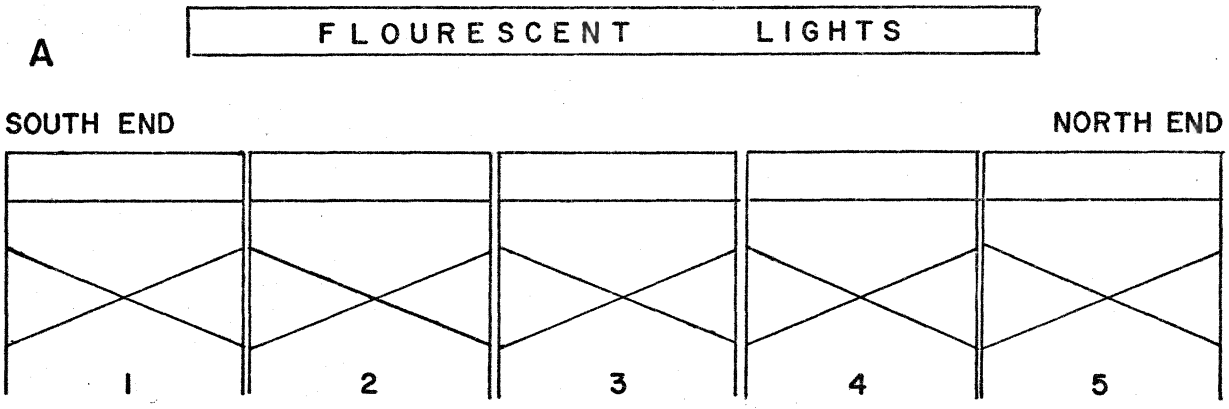
Stock populations of Meloidogyne incognita were propagated on tomato varieties Tiny Tim and Glamour in greenhouses at the Canadian Department of Agriculture Research Station, Vineland Station, Ontario. Chopped up severely infected roots were thoroughly mixed with a quantity of potting soil (top soil/peat/sand, 1/1/1 by volume). This mixture was placed in 12.5 cm clay pots and a single seedling planted in each pot. These stock plants were grown for at least six months, to allow for multiple reinfestations to occur during the course of several life cycles. After twelve months, stock plants were replaced by young seedlings using the soil and

Figure 4. (A) Arrangement of experimental plants in the greenhouse. There were five benches centred under a bank of fluorescent lights.

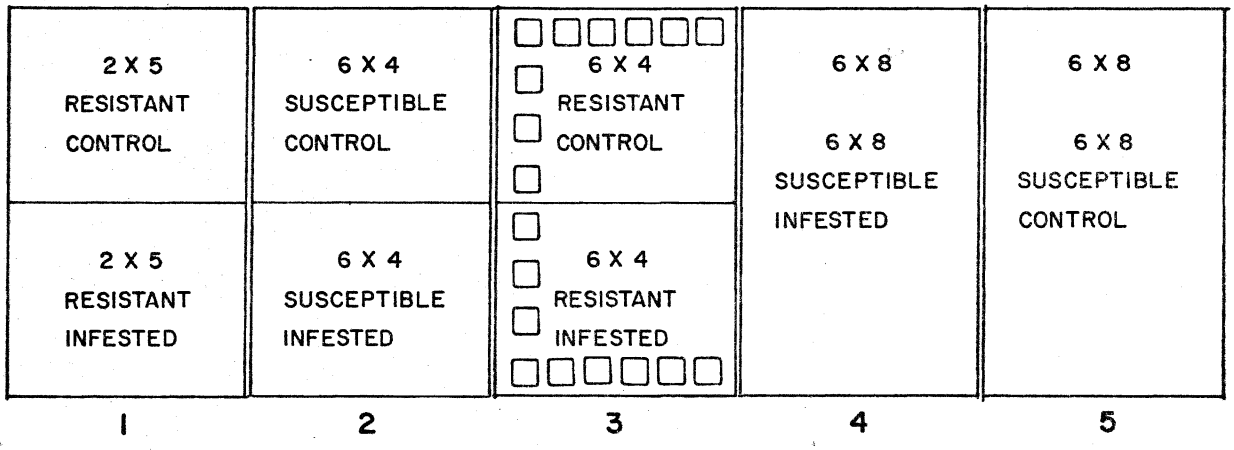
(B) Arrangement of plants of experiments 3 (susceptible) and 4 (resistant). This run of experiment 3 consumed 60 plants, 30 controls and 30 infested. Experiment 4 had 14 runs which consumed 56 plants, 28 control and 28 infested. All the plants on benches 2 and 3 were used for the first 12 runs of both experiments while the last few experimental runs were obtained from plants grown on benches 1, 4 and 5. There was an excess of plants left after completion of these experiments. Experiments 1 and 2 were performed independently with 250 plants consumed in experiment 1 and 128 plants consumed in experiment 2 (see experimental design). The susceptible plants of these experiments were arranged so that half of each bench contained infested and half control plants.

(C) Arrangement of plants of experiments 5 (susceptible) and 6 (resistant). Note that individual benches were not divided into an infested and control half as in other experiments. Squares on bench 3 of figures B and C represent potted plants and indicate their spacing according to the numbers indicated per bench.

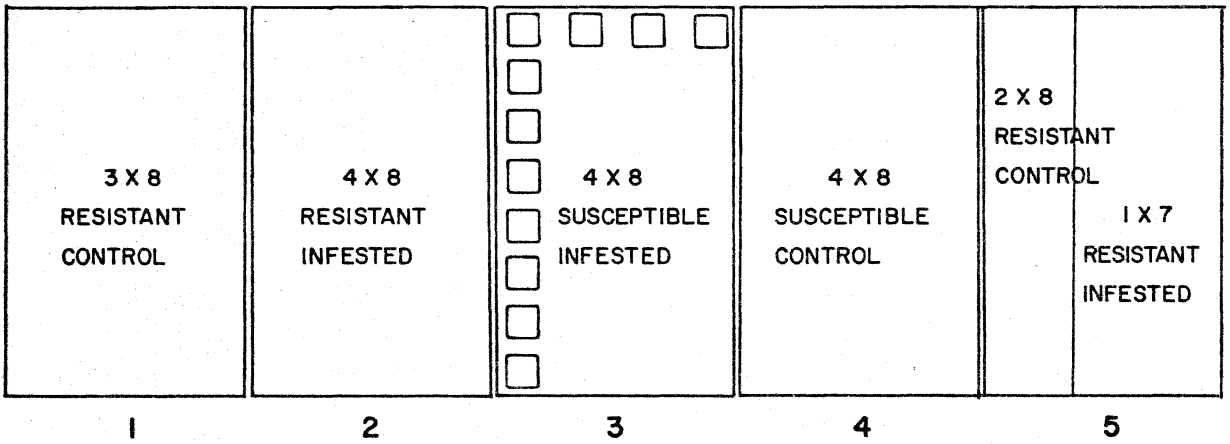
A


FLOURESCENT LIGHTS
SOUTH END
NORTH END
1
2
3
4
5

B


2 X 5
RESISTANT
CONTROL
6 X 4
SUSCEPTIBLE
CONTROL
6 X 4
RESISTANT
CONTROL
6 X 8
6 X 8
SUSCEPTIBLE
INFESTED
6 X 8
6 X 8
SUSCEPTIBLE
CONTROL
2 X 5
RESISTANT
INFESTED
6 X 4
SUSCEPTIBLE
INFESTED
6 X 4
RESISTANT
INFESTED
1
2
3
4
5

C


3 X 8
RESISTANT
CONTROL
4 X 8
RESISTANT
INFESTED
4 X 8
SUSCEPTIBLE
INFESTED
4 X 8
SUSCEPTIBLE
CONTROL
2 X 8
RESISTANT
CONTROL
1 X 7
RESISTANT
INFESTED
1
2
3
4
5

roots of the old plants as described above. This was necessary since the old stock plants were moribund.

Stock plants were denied water for twenty-four hours prior to harvest in order to facilitate the separation of roots from soil. Plants were removed from their pots and the roots freed from the bulk of the soil by gentle manual agitation and manipulation, conserving the soil for new stock cultures. Roots were transferred to a large bucket of water, allowed to soak for a few minutes and then gently agitated to remove most of the remaining soil. The washing procedure was completed with running tap water. Cleaned roots were chopped up thoroughly with scissors and placed on 10 cm diameter flat circular screens. These were fitted onto the top ends of funnels and placed on a rack in a mistifier so that the bottom end of the funnel drained into a 680 ml Mason jar. The mistifier provided a fine spray of water every five minutes which settled over the roots, collected and drained down the funnels to the Mason jars. Under these conditions, egg masses at the surface of the roots hatched and the emerging second stage larvae were swept into the collecting jars. Being more dense than water, they settled to the bottom whilst excess water ran out of the open tops. Harvesting proceded for two weeks after which time the jars were left to settle for twenty-four hours.

The collected nematodes were concentrated by drawing off the supernatant fluid using a piece of glass tubing bent fish-hook fashion and attached in series to a large erlenmeyer flask and vacuum line. The glass hook was manipulated so as to draw from just below the surface of the supernatant fluid to prevent perturbation of the nematodes on the bottom of the Mason jars. The concentrated nematode preparation was pooled, stirred thoroughly and assayed for nematodes. Nematode counts were made by removing five,

one ml aliquots via a wide-mouthed 5 ml pipette to five counting chambers each with a 10 mm x 10 mm counting grid. Each counting chamber had been pretreated with a drop of surfactant (Tween 20) to reduce surface tension. The number of nematodes in each one ml aliquot was estimated by counting numbers within the ten, 1 mm x 1 mm squares on each diagonal. The average of these five, one ml counts was used to calculate the total number of nematodes collected.

4-4. Infestation of Plants with Nematodes

Plants were infected in the laboratory and then returned to the greenhouse. The method of infection was that suggested by J. W. Potter (Canada Department of Agriculture, Vineland Station, Ontario). Concentrated nematode extract was diluted so that the total nematodes collected could be distributed equally to plants in 10 ml aliquots. Plants in Experiment 1 received 2,000, Experiment 2 10,000, Experiments 3 and 4 18,000, and Experiments 5 and 6 17,000 Meloidogyne incognita second stage larvae per plant. The inculum concentrate was continually stirred with a magnetic stirrer (PC351, Corning) to ensure even distribution of the nematodes. The inoculum was drawn into a 10 ml plastic syringe (Plastipak, Becton-Dickinson and Company, Mississauga, Ontario) fitted with a 3.8 cm 18 gauge hypodermic needle. The needle was thrust deep into the sand in each corner of the pot and 1 ml of inoculum injected at each location. The remaining six ml were evenly distributed on, or just under the surface of the sand.

Water was withheld from the plants twelve to twenty-four hours prior to infestation. Nematodes can travel through the sand and penetrate the roots best under damp but not wet conditions. (J. W. Potter, personal

communication). Care was taken to water plants individually for the three days following infestation to maintain these favourable conditions and "water in" the larvae. Both susceptible and resistant varieties were treated in this manner. Control plants received a 10 ml aliquot of distilled water applied as described.

4-5. Gas Determinations

A. Extraction of root gases

The method of Beyer and Morgan (1970) for extracting gases of vegetative tissue was employed with certain modifications. The technique involved collecting internal root gases by exposing the roots to a vacuum. The apparatus used for these extractions is shown in Figure 5. It consists of an evacuation chamber (25 cm glass dessicator), a gas collection vessel, a mercury manometer and an aspirator. The evacuation chamber is connected via rubber vacuum hosing to a 25 cm mercury manometer (Technical Services, Brock University) and the aspirator. The chamber is filled with a saturated solution of ammonium sulphate. The collection vessel resembles an inverted beaker (1 liter), the bottom of which is drawn out funnel-like to terminate in a small opening. Over this opening is secured a sleeve type neoprene rubber septum (Chromatographic Specialities Limited, Brockville). This vessel was submerged under the liquid so that it was entirely filled, any air bubbles that had collected under the septum were withdrawn using a ten ml gas-tight syringe (Hamilton #1001). Saturated ammonium sulphate was used in place of water since the solubility of ethylene in this solution is negligible (Beyer and Morgan, 1970).

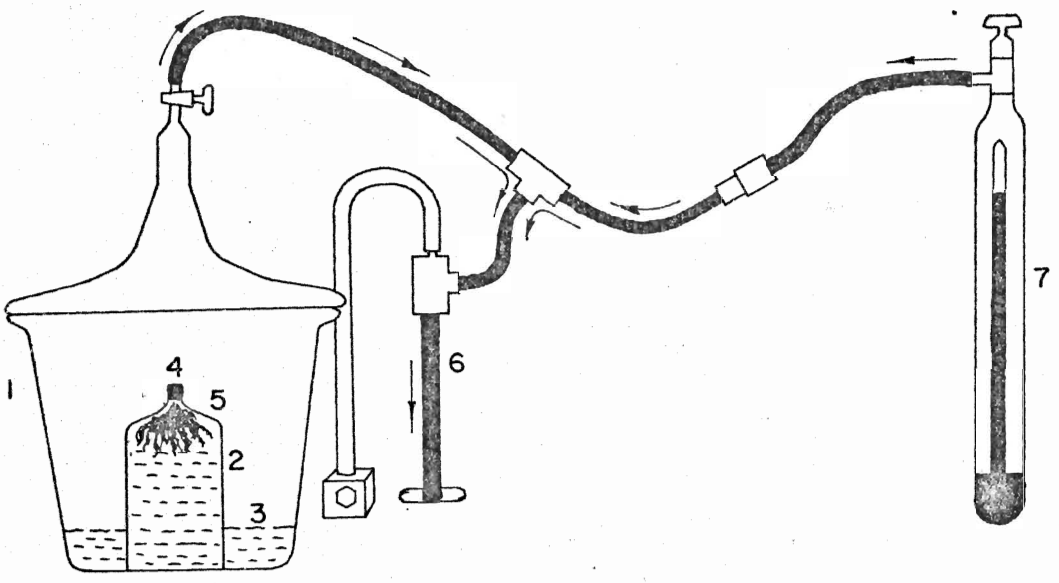
The following gas extraction procedure was used. Plants were taken

to the gas chromatography laboratory, and the stem cut off at the level of the sand. The potted root was inverted and the side of the pot struck against the side of the sink to free the entire root system. This was immersed in a plastic container filled with tap water and gently rotated, the sand falling easily from the roots. Running tap water was used to remove any remaining sand. The cleaned roots were submerged in a solution of surfactant (0.01% Tween 20), rotated gently and transported in this solution to the collection apparatus. Roots were then transferred to the ammonium sulphate solution, submerged, gently rotated to free adhering air bubbles and slipped under the open bottom of the collection vessel. Since the liberated gases expand considerably under reduced pressure, about two-thirds of the liquid around the collection vessel was siphoned off to prevent the collection vessel from tipping over during evacuation. The dessicator top was replaced and with the valve to the manometer closed the line was connected to the aspirator and evacuation begun. After five minutes, the stopcock to the manometer was opened and development of the reduced pressure observed.

The entire procedure, from cutting the stem to commencement of evacuation required two and a half minutes: 45 seconds root wash, 15 seconds in surfactant, 45 seconds in loading the collection vessel and 45 seconds to seal the chamber and connect the lines.

Gases were extracted in Experiment 1 for a 45 minute period using a single gas extraction apparatus. A second apparatus was introduced for later experiments which enabled two samples to be extracted simultaneously. The mercury manometers were introduced by run 15 of Experiment 2. Gases were extracted from the roots of Experiment 2 plants for fifteen minutes up to run 15, after which time gases were extracted until a partial pressure

Figure 5. Apparatus used in the extraction of tomato root gases. A 25 cm glass dessicator (1) was filled with saturated ammonium sulphate solution (3). The gas collection vessel (2) had an open bottom with a funnel-like top terminating in a small opening over which was secured a neoprene septum (4). Roots (5) from which gases were to be extracted were placed inside this vessel. Once sealed the glass dessicator was connected via rubber vacuum hosing (solid lines) to an aspirator (6) and mercury manometer (7). Arrows indicate the direction of air flow during evacuation.


1
2
3
4
5
6
7

of 2.0 to 3.0 cm Hg was reached. This generally required fifteen minutes. The latter procedure was employed for Experiments 3, 4, 5 and 6. At the end of each extraction the stopcock to the evacuation chamber was closed and the partial pressure and time of evacuation recorded. Air at atmospheric pressure was then carefully readmitted to the evacuation chamber.

By means of a 1.0 ml gas-tight syringe, an aliquot of the collected gas sample was withdrawn and immediately injected into the gas chromatograph. The volume of the aliquot removed was 1 ml or that volume of gas which could be readily taken without drawing up any liquid. The total volume of gas was obtained by withdrawing the remaining gas and some liquid, reading the gas volume and summing this portion with the previous aliquot withdrawn.

It was necessary to establish at which point in the extraction process further collection of root gases became redundant. Collection of root gases in Experiment 1 proceded for forty-five minutes per sample. Observations were made over the course of this experiment on the emergence of gas bubbles from the roots during evacuation. At the termination of Experiment 1, experiments were performed to establish a time course for the collection of root gases.

Susceptible (var. Vendor) and resistant (var. Anahu) tomato plants (116 days old) were used in a total of seven experiments (four with susceptible plants, three with resistant plants). For each experiment, two root systems were used. Extraction of gases proceded for a ten minute interval at which point the aspirator was shut down and the volume of gas collected measured. Extraction was reinitiated for an additional ten minute interval and the procedure repeated six times to cover a total of sixty minutes of gas collection time. The sum of gas volumes collected at each time interval was recorded.

The drop in partial pressure of the collection chamber during the course of gas collection was measured with a 30 cm mercury manometer (Technical Services, Brock University) placed in series with the evacuation chamber and aspirator of each gas collection apparatus. This enabled direct measurement of the partial pressure of the chamber at any point in the course of evacuation. For each of the four plant samples of run 15, the partial pressure in the gas collection chamber was recorded at regular intervals. Gas collection was terminated when a partial pressure of 2.0 to 3.0 cm Hg was reached.

4-5. B. Preparation of Gas Standards

(i) Collection of a pure gas sample

All ethane and ethylene analyses conducted on the gas chromatograph were under the same operating conditions, those of maximum sensitivity (range 1, attenuation 1). Appropriate standards were selected so that the highest concentration gave close to full scale pen deflection on the 25 cm strip chart redorder. Once this was established, a range of standards was prepared by a volumetric dilution technique. A triple neck flask was alternately evacuated to about 10 mm Hg and filled with CP grade ethylene or ethane five times (G. R. Finlay, Brock University). One neck of the flask was connected via rubber hosing and glass tubing to a motorized vacuum pump (Edwards High Vacuum Limited, Crawley, England). The middle neck of the flask was sealed with a sleeve type neoprene septum and the third attached to the cylinder of ethylene via a modified regulator which indicated both positive and negative pressures (Union Carbide Canada Limited, Toronto). This allowed the partial pressure of the flask and lines to be read during

the course of evacuation and when bleeding gas back into the flask.

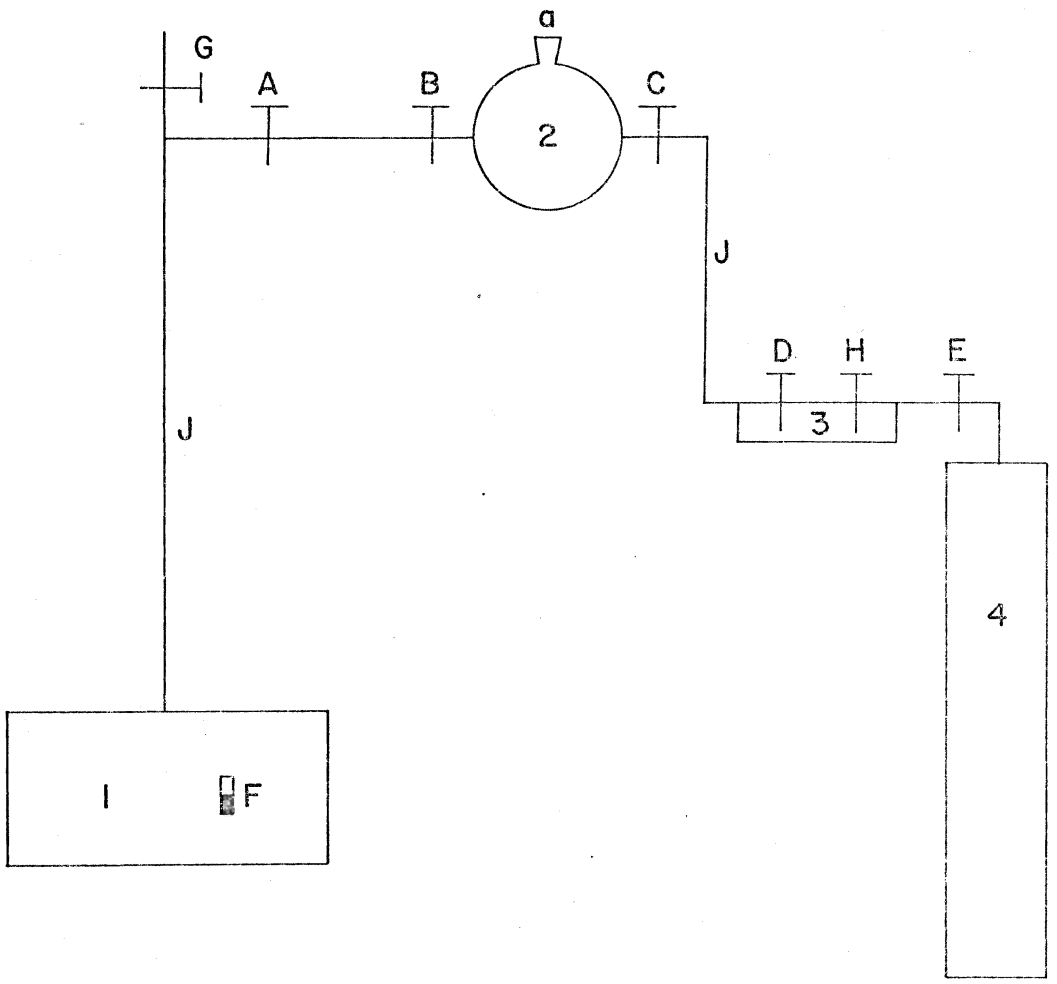
Evacuation of the flask proceded as follows: Stopcock G was closed and kept closed throughout all operations. Switch F to the vacuum pump was switched on and stopcocks A, B, C and D opened to evacuate the lines and collection flask. With valve E closed, valve H was then opened to evacuate the regulator, enabling the partial pressure in the flask and lines to be read. The pumping procedure continued for two minutes which was adequate to ensure a partial pressure in the range of 10 mm mercury.

Gas was bled into the system as follows. Stopcock B and valve H were closed and switch F turned off. Valve E was then opened briefly to flood the regulator with gas and then closed down again. Slowly opening valve H allowed gas back into the lines and flask at a controlled rate until a positive pressure of two to three cm mercury on the pressure indicator was obtained. At this point the pump was turned on (Switch F) Stopcock B opened and another repetition of evacuation begun. The procedure was repeated five times to obtain a sample of pure gas.

Replacing the vacuum in the flask with ethylene resulted in 750 parts ethylene to 10 parts residual air. Numerically 10/760 x 100% = 1.4% of the gas sample after a single run was residual air. A repetition of the procedure would reduce the residual air by a similar factor and after two repetitions the percentage of air in the flask would be 0.03%. Five such repetitions would result in this fraction becoming vanishingly small. The purity of gas collected in the triple neck flask was limited by the purity of the gas purchased from the supplier (i.e., CP grade ethylene 99.5% guaranteed purity, ethane 99.0% guaranteed purity). After the fifth repetition was completed, the internal gas pressure was allowed to equilibrate to atmospheric pressure by rapidly opening Stopcock B or C. Pure

Figure 6. Apparatus used in obtaining a high purity sample of ethane or ethylene at atmospheric pressure.

A cylinder of gas (4) was connected to a triple neck flask (2) in which the gas was collected. The system was alternately evacuated with a mechanical pump (1) and flushed with gas to ensure a sample of high purity gas was obtained. G, A, B, D--stopcocks; C--needle valve; E--gas cylinder valve; F--pump 'on-off' switch; J--rubber vacuum hosing; 3--regulator and pressure indicator; a--middle neck of triple neck flask sealed with a neoprene septum; H-- regulator valve.


a
G
A
B
2
C
J
D
H
E
3
J
4
I
F

gas samples of ethane were obtained as described here for ethylene. Barometric pressure and temperature for each set of standards were recorded.

(ii) Volumetric Dilutions to Obtain Final Standard Concentrations

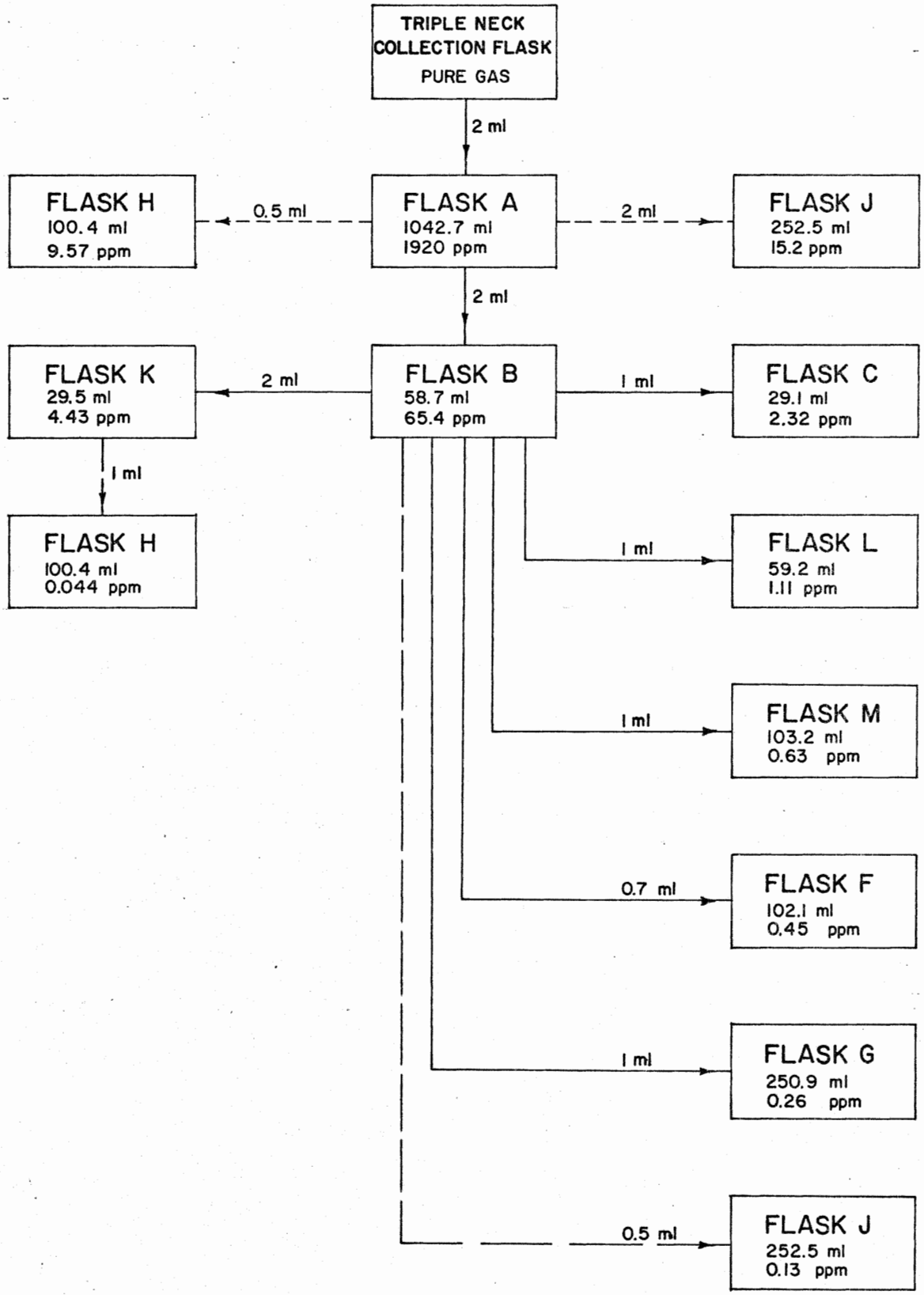
Standards were prepared immediately using volumetric dilutions of the pure gas sample into flasks of various volumes of air sealed with neoprene septa. A gas tight syringe was used to inject aliquots across the septa. Determinations of the volume of the flasks were made by measuring the weight of water each held assuming a density of water within the temperature range encountered of 1.00 g/ml (Weast, 1972). Ethylene standards of concentrations 0.044, 0.13, 0.26, 0.45, 0.63, 1.11, 2.25 and 4.43 µl/liter were prepared with concentrations of 9.57 and 15.2 µl/l replacing the lowest two concentrations for Experiments 5 and 6 (Figure 7).

For a final concentration of 0.26 µl/l the following dilutions were made. Two ml of pure gas taken from the triple-neck flask were injected into vessel A of 1042.7 ml volume to give a concentration of 1920 µl/l. Two ml of this preparation were then injected into Flask B of volume 58.7 ml yielding a concentration of 65.4 µl/l. Finally, 1 ml of gas from Flask B was injected into Flask G of volume 250.9 ml to yield 0.26 µl/l.

A total of 7.2 ml of gas was withdrawn from Flask B in the process of serial dilutions (Figure 7). Since withdrawing this amount of gas from a container of 58.7 ml volume would result in inaccuracy due to slightly negative partial pressure, the contents of Flask B were prepared again after half the standards had been prepared from it.

Gas samples were injected into the gas chromatograph using the following procedure. Gas standards were shaken vigorously to ensure

Figure 7. Dilution sequence used in preparation of gas standards. Solid lines represent dilutions made for standards used in all experiments while the broken lines (— —) represent standard dilutions used in experiments 1-4 only and the dashed(---) line, experiments 5 and 6 only. Concentrations are given in parts per million which is equivalent to microlitres per litre.


TRIPLE NECK
COLLECTION FLASK
PURE GAS
2 ml
FLASK A
1042.7 ml
1920 ppm
0.5 ml
FLASK H
100.4 ml
9.57 ppm
2 ml
FLASK J
252.5 ml
15.2 ppm
2 ml
FLASK B
58.7 ml
65.4 ppm
2 ml
FLASK K
29.5 ml
4.43 ppm
1 ml
FLASK C
29.1 ml
2.32 ppm
1 ml
FLASK H
100.4 ml
0.044 ppm
1 ml
FLASK L
59.2 ml
1.11 ppm
1 ml
FLASK M
103.2 ml
0.63 ppm
0.7 ml
FLASK F
102.1 ml
0.45 ppm
1 ml
FLASK G
250.9 ml
0.26 ppm
0.5 ml
FLASK J
252.5 ml
0.13 ppm

mixing. The syringe was flushed out with air and then flushed with about 0.5 ml of the standard to be run. One ml of the lowest concentration was removed from its flask and injected into the column; the needle was then replaced into the standard being analysed. This procedure eliminated any errors due to diffusion of air into the needle tip. Analyzing standards of lowest concentration first ensured that no gas at a higher concentration was transferred in the needle tip to a more dilute standard. Standards were analyzed immediately after preparation. Each gas standard was analyzed at least three times (more if peak size was unusually variable) for every set of standards. Over the course of an experiment, a set of standards was prepared and analysed two or three times, recording the barometric pressure and air temperature with each set.

4-5. C. Analysis of Gas Samples

Gases were analyzed on a Hewlett-Packard 5700A Gas Chromatograph equipped with a flame ionization detector. Eluent gases were monitored by a Fisher Recordall Series 5000 electronic recorder and integrator. Operating conditions for maximum sensitivity were selected (range 1, attenuation 1). The oven was operated isothermally at 55°C, the injector port at 100°C and the detector at 150°C. A 183 cm x 0.64 cm teflon (or glass) column packed with Porapak Q mesh range 80/100 (Waters Associates, Milford, Massachusetts, U. S. A.) was used. New columns were conditioned by heating to 250°C for three hours with a slow flow of nitrogen carrier gas. Carrier gas flows of 60 ml per minute and 30 ml per minute were used for Experiments 1, 2, 3 and 4, and Experiments 5 and 6, respectively. Hydrogen was supplied to the detector at rates of 60 ml per minute (Experiments 1, 2, 3 and 4) and 35 ml per minute (Experiments 5 and 6). Air flow to the detector was

constant for all experiments at 240 ml per minute. Improved reproducibility in peak size of lower concentrations was realized with the lowered flow rates.

Tests were conducted to determine whether a direct proportionality between recorder response and volume of gas sample injected existed. A standard dilution of 2.34 µl/l ethylene was prepared and volumes of 100, 200, 300, 400, 500, 700 and 1000 µl were injected into the gas chromatograph. The recorded peaks were integrated to determine their areas and compared to the corresponding volumes of gas sample.

4-5. D. Identification of Gases Extracted from Roots

Identification of the peaks obtained on analysis of an aliquot of sample gas was undertaken.

1. The mean retention time of each sample peak was computed. Lecture bottles of known gas types were used to prepare authentic gas species diluted in air. One ml aliquots of these samples were then injected into the gas chromatograph. Mean retention times for ethane, ethylene and methane were determined and compared to the retention times of gases extracted from roots.

2. Sample gases were exposed to mercuric perchlorate, a reagent that specifically complexes ethylene and other olefins. Twenty-five ml of this reagent was placed in a Warburg flask with its sidearm stoppered. The top of the flask was fitted with a neoprene sleeve-type septum and an aliquot of sample gas injected. The vessel was shaken well to ensure that any olefins in the sample complexed with the mercuric perchlorate. The side arm stopper was then removed and the uncomplexed gases displaced by air. An equal volume of 4 M lithium chloride was added to the side arm

chamber, the sidearm restoppered and the vessel tilted to mix the two reagents. Reaction with the salt solution results in the release of the olefins. A one ml aliquot of the gases in the vessel was removed with a gas-tight syringe and immediately injected into the gas chromatograph.
3. Standard preparations of pure ethylene and samples of root gases were injected into a gas chromatograph (Q4E104)-mass spectrograph (Associated Electric Industries, MS30) connected via a Watson-Biemann glass frit or silica membrane separator for further identification of sample gases.

4-6. Growth Measurements

Measurements of growth were made by recording stem heights, top weights and root weights. Plants to be analyzed for root gases were first measured for stem height using a straight rule and then cut off at the level of the potting soil. While the roots were being prepared for gas extraction, the upper portions (stem and leaves) were put aside until the gas extraction was underway. Top weights were then measured using a top-loading Mettler P1200 balance (Fisher Scientific Company). After gas collection was completed, roots were removed from the evacuation chamber and washed briefly under running tap water to remove the salt solution.

4-7. Preservation and Staining of Root Material

Roots were preserved and stained at the end of each run by immersing the root material in 0.1% boiling lactophenol-Cotton Blue for two minutes (Goodey, 1951). After cooling, roots were placed in open containers, labelled and stored in ambient air until the conclusion of all experiments.

4-8. Measurements of Galling

A modification of the procedure of Townshend and Baenziger (1976) was used to measure the extent of galling. Stained and preserved roots were examined by eye and under a dissecting microscope (A.U.S. Jena). An estimate of the precent of the total root mass composed of galls was recorded and the mean percentage value determined for each run. Galling was classified on a scale of 1 to 4 according to the mean calculated percentage where 1 represents 0%, 2 less than 25%, 3 25% to 49%, 4 greater than 49%.

4-9. Measurements of the Rate of Ethylene Production

An apparatus was designed to measure the rates of ethylene production by the plants. In principle, the apparatus consisted of a container which enclosed the plants and through which air passed to an ethylene trap. A chamber was designed and built of plexiglass with dimensions 45 cm deep x 45 cm wide x 61 cm high. The joints were sealed with air-tight glue and the container reinforced around the vessel at mid height. The movable top was 5.0 mm plate glass with a rubber strip around the perimeter of the lower surface 2.0 cm from the edge. The top was placed over the chamber and the rubber stripping sealed the system when tightened down with eight G-clamps. Air was supplied to the chamber via a compressor (The Jas. Morrison Brass Mfg. Co. Ltd., Toronto) and a Hoke Gyrolok 3812 G 4B needle valve. An air flow of 3,300 ml per minute was measured with a Matheson (Whitby, Ontario) model 7254 flow meter. Effluent air from the vessel was passed through a dry ice-acetone water trap and indicarb (10-20 mesh) to remove CO_2. Ethylene was then collected in a copper U-tube 6.4 mm in internal diameter with arms about 10 cm long. On the open ends

of these were 6.4 ml Swagelock fittings (Chromatographic Specialities Ltd., Brockville, Ontario). The U-tubes were packed with various ethylene trapping agents: 1. they were loosely packed with glass angel hair and immersed in liquid nitrogen (Abeles, 1973), 2. packed with 80 mesh silica gel and immersed in dry ice-acetone (Phan, 1976), 3. packed with 4 Å molecular sieve and left at room temperature (Frieser and Frieser, 1967).

The U-tubes were attached to the gas chromatography apparatus while still immersed in their appropriate baths and then heated to release the bound gas into the sealed trap. The liquid nitrogen technique required warming the U-tubes to room temperature, the silica gel trap required heating to 60°C in a water bath and the molecular sieve trap required heating to 350°C in molten solder (60°lead, 40°tin, Mastercraft Ltd., Toronto).

After release of the trapped ethylene, needle valves were manipulated so as to direct the flow of carrier gas through the U-tube and sweep the ethylene into the column.

Various operating conditions of the gas chromatograph were employed to establish the optimal settings for sensitivity and resolution of ethylene peaks. Standard preparations of ethylene or ethane were used with these traps to establish the retention times of the gases released from the U-tubes.

4-10. Statistical analysis

A. Confidence Intervals for Means

Confidence limits about the mean were calculated from the formula:

$$\bar{z} \pm t\left(\frac{s}{\sqrt{n}}\right)$$, where: $\bar{z}$ = mean of sample

t = critical t for 95% significance at n - 1 degrees of freedom

s = standard deviation of sample

n = sample size

(from Campbell, 1967)

EXAMPLE 1. Ethylene Standard Curve for Experiments 1, 2, 3, 4

Standards of concentration 2.25 µl/l analyzed eleven times at various dates yielded the following data:

$\bar{z}$ = mean peak size = 35.5 integrator counts

x = standard deviation = 3.59

degrees of freedom = 10

t critical at 95% significance = 2.228

confidence interval = $t\left(\frac{s}{\sqrt{n}}\right) = \pm 2.4$

confidence limits of mean:

upper limit = 37.9 integrator counts

lower limit = 33.1 integrator counts

4-10B Linear Regression Analysis

(i) Growth Data

The relationship between the independent variate x and the dependent variate y was tested for significant correlation with a linear regression analysis. The method of least squares as defined by Mendenhall (1975) was used.

$$y = \hat{\beta}_0 + \hat{\beta}_1 x$$

where $\hat{\beta}_1 = \text{slope} = \dfrac{SSxy}{SSx}$

$$\hat{\beta}_0 = y \text{ intercept} = \bar{y} - \hat{\beta}_1 \bar{x}$$

and, $$SSxy = \text{sum of squares for } xy = \sum_{i=1}^{n} x_i y_i - \frac{\left(\sum_{i=1}^{n} x_i\right)\left(\sum_{i=1}^{n} y_i\right)}{n}$$

$$SS_x = \text{sum of squares for } x = \sum_{i=1}^{n} x_i^2 - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n}$$

Linear regressions were computed for growth data by first calculating the differences of the dependent variate y, control minus infested and correlating these differences to the independent variate x (time).

EXAMPLE 2. Experiment 3. Root Weight versus Time

Differences in root weights, control minus infested are listed under column y, the dependent variate. X, the independent variate represents the time of each experimental run from time zero (commencement of experiment).

Run	x (Time in days)	y (Root weights C - I in g)	x^2	y^2	xy
1	0.25	1.5	0.0625	2.25	0.375
2	1	-0.6	1	0.36	-0.6
3	2	-0.4	4	0.16	-0.8
4	4	-0.8	16	0.64	-3.2
5	6	-2.7	26	7.29	-16.2
6	9	-2.4	81	5.76	-21.6
7	11	1.4	121	1.96	15.4
8	13	-1.3	169	1.69	-16.9
9	15	-2.3	224	5.29	-34.5
10	19	-7.0	361	49.00	-133
11	21	-4.5	441	20.25	-94.5
12	23	-6.9	529	47.61	-158.7
13	25	-7.3	625	53.29	-182.5
14	27	-14.5	729	210.25	-391.5
15	30	-11.0	900	121.00	-330.0

$N = 15$

$$\sum_{i=1}^{n} x = 206.3$$

$$\sum_{i=1}^{n} y = -58.8$$

$$\sum_{i=1}^{n} x^2 = 4{,}238.06$$

$$\sum_{i=1}^{n} y^2 = 526.8$$

$$\sum_{i=1}^{n} xy = -1368.2$$

(a) Calculate $\hat{\beta}_1$, the slope

$$\hat{\beta}_1 = \frac{SSxy}{SSx} = -0.40$$

$$SSxy = -559.53$$

$$SSx = 1,400.75$$

(b) Calculate $\hat{\beta}_0$, the y-intercept

$$\hat{\beta}_0 = \bar{y} - \hat{\beta}_1\bar{x}$$

$$\bar{y} = \frac{\sum_{i=1}^{n} y_i}{n} = -3.92$$

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n} = 13.75$$

Therefore, $\hat{\beta}_0 = 1.57$

Thus, the equation of line of best fit is

$$y = 1.57 - 0.40x$$

(ii) Gas Concentration Data

A similar Linear Regression analysis of gas data was computed. The dependent variate y, gas concentration, was correlated to x, time, using a percentage difference basis. The largest concentration of an experimental run whether control or infested was designated the numerator, with the smaller the denominator and the percentage of one to the other calculated. Percentages

so calculated with control concentrations larger were designated positive and the inverse (i.e., infested greater than control) designated negative. If control and infested gas concentration for a particular run were identical, then they would equal 100%. One hundred percent was subtracted from each calculated value to yield the percentage difference from zero, either positive (control larger) or negative (infested larger).

EXAMPLE 3. Experiment 2. Run 1, Day 0.08 (2 hours)

ethylene concentration control = 0.065 ng C_2H_4 per gram root

ethylene concentration infested = 0.066 ng C_2H_4 per gram root

Percentage difference

$$\frac{0.066 \text{ ng } C_2H_4/\text{gram root}}{0.065 \text{ ng } C_2H_4/\text{gram root}} \times 100\% - 100\% = 2\%$$

Since infested was the larger value and the numerator, the difference is designated negative.

Thus the percentage difference is -2%

These percentage differences were then correlated to the independent variate x, time, as outlined in the growth data example.

All other linear regression analyses performed incorporated the data as recorded experimentally without further arithmetic conversions or operations.

(iii) Test for Significant Correlation:

With each linear regression analysis a coefficient of correlation r was computed:

$$r = \frac{SSxy}{\sqrt{SSx SSy}} \qquad \text{(Mendenhall, 1975)}$$

The value obtained was compared to the critical value at 95% confidence and

n - 2 degrees of freedom for a two-tailed test (Murdoch and Barnes, (1970)). A value of r greater than the critical value indicated that a significant correlation existed.

EXAMPLE 4. Experiment 3. Root Weights versus Time.

From the table of example 2, solve for r:

SSxy = -1368.23

SSy = 526.8

SSx = 4238.06

$$r = \frac{SSxy}{\sqrt{SSxSSy}} = \frac{-1368.23}{\sqrt{(4238.06)(526.8)}} = -0.92$$

critical r for 95% confidence and 13 degrees of freedom = 0.59

decision: There is a significant negative correlation of root weight differences, control versus infested, with time; or equivalently infested root weights increased significantly in a linear manner over controls.

The numerator here is the same as that for $\hat{\beta}_1$ the slope, so that a negative slope indicates a negative correlation and positive slope a positive correlation. Logically, when the slope equals zero, there is no linear correlation since r must also equal zero. Further if there is a significant linear correlation, it follows that the slope will be significantly different from zero (Mendenhall, 1975)[1]

---

[1] A slope significantly different from zero can be tested using $t = \frac{\hat{\beta}_1 - \hat{\beta}_{10}\sqrt{SSx}}{s}$ where $\hat{\beta}_{10}$ is the slope of the bivariate population data and $\hat{\beta}_1 = 0$ for the null hypothesis. Calculations using this formula gave decisions identical to those obtained with the coefficient of correlation and was thus abandoned as redundent for further calculations.

(iv) Test for a Zero Intercept

Standard curve 1 for ethylene gas concentrations provided a particular problem in that some experimental values were less than the theoretical y-intercept computed by linear regression analysis (see results). To overcome this problem, it was assumed that the y-intercept was actually zero and the line of best fit was defined by $y = \hat{\beta}_1 x$ (Bliss, 1967). To test whether the line so defined fitted the data within acceptable limits of confidence, the following calculations were done.

Workform 13-8. Test of a Zero Intercept

Term	D.F.	SS and SP			Slope	Reduced Estimates			
		x^2	xy	y^2		D.F.	SS	Ms	F
Intercept	1	$C\bar{x}$	Cxy	$C\bar{y}$	$\beta_1{}^2-\beta_0{}^2$	1	$C\bar{y}-\beta_1{}^2+\beta_0{}^2$	A_0	A_0/S^2
Scatter	N-1	$[x^2]$	[xy]	$[y^2]$	$\beta_0{}^2$	N-2	$[y^2]-\beta_0{}^2$	S^2	$\beta_0{}^2/S^2$
Total	N	Σx^2	$\Sigma(xy)$	Σy^2	$\beta_1{}^2$	N-1	$\Sigma y^2-\beta_0{}^2$	$S_0{}^2$	$\beta_1{}^2/S_0{}^2$

$$C\bar{x} = \frac{(\Sigma x)^2}{N} \qquad Cxy = \frac{\Sigma x \Sigma y}{N} \qquad C\bar{y} = \frac{(\Sigma y)^2}{N}$$

(from Bliss, 1967)

The scatter row represents the line of best fit by the method of least squares which has both a slope and y-intercept. The total row is the line of best fit constrained to pass through the origin. The difference between these lines A_0 is the sum of the squares resultant of fitting an intercept and slope to the data. The F value A_0/S^2 tests the null hypothesis that the true intercept is zero by comparison of this figure to the critical F values at the appropriate level of significance and degrees of freedom.

EXAMPLE 5. Ethylene Standard Curve 1.

x Concentration of standard (µl/l)	y mean peak size (counts)	xy	x^2	y^2
0	0	0	0	0
0.04	7.9	0.348	0.0016	62.41
0.13	10.7	1.391	0.0169	114.49
0.26	12.5	3.263	0.0676	156.25
0.45	13.1	5.869	0.2025	171.61
0.63	21.4	13.487	0.3969	457.96
1.11	30.7	34.077	1.2321	942.49
2.25	35.5	79.875	5.0625	1260.25
4.43	68.6	303.898	19.6249	4705.96
Totals				
9.30	200.4	442.203	26.6050	7871.42

Using these calculations workform 13.8 was completed

Term	D.F.	SS and SP			Slope	Reduced Estimates			
		x^2	xy	y^2		D.F.	SS	MS	F
Intercept	1	9.61	207.20	4462.24	2099	1	363	363	16.29
Scatter	8	17.00	235.00	3409.18	3253	7	156	22.29	145.9
Total	9	26.61	442.20	7871.42	7352	8	519	64.88	113.3

$$\text{slope} = \frac{\Sigma(xy)}{\Sigma x^2} = \frac{442.20}{26.61} = 16.62$$

equation of line of best fit: $y = 16.62\ x$

F value to test the fit of zero intercept line = A_0/S_2= 16.29

Critical F value at 95% confidence = 5.32

Decision: Constraining the line of best fit to pass through the origin fits the data to a high degree of confidence and is therefore an acceptable assumption.

4-10. C. Paired Sample t-Test

This test was employed on data where no significant correlation existed as determined by linear regression analysis. The test calculated an interval estimate for the difference between the population means of control and infested treatments and provided a significance test on the null hypothesis that this difference was zero

EXAMPLE 6. Experiment 2. Root Weights Control versus Root Weights Infested.

Run	x	y	z	z^2	Run	x	y	z	z^2
1	11.8	12.2	-0.4	0.16	11	12.9	13.3	-0.4	0.16
2	9.8	10.8	-1.0	1.0	12	10.8	13.3	-2.5	6.3
3	9.9	12.2	-2.3	5.3	13	13.7	12.7	1.0	1.0
4	10.2	12.2	-2.0	4.0	14	12.4	14.2	-1.8	3.2
5	10.6	12.2	-1.5	2.3	15	16.4	18.1	-1.7	2.9
6	12.3	12.0	0.3	0.09	16	15.3	16.5	-1.2	1.4
7	11.5	13.3	-1.8	3.2	17	6.8	8.7	-1.9	3.6
8	9.6	11.6	-2.0	4.0	18	8.6	9.4	-0.8	0.64
9	12.4	12.2	0.2	0.04	19	10.3	14.3	-4.0	16.0
10	10.6	14.3	3.7	13.7	TOTALS			-27.5	68.99

x = Root weight control (g), y = root weight infested (g), z = x - y

Mean difference = $\bar{z} = -1.45$

Test for significance,

$$\text{observed } t = \frac{\bar{z}}{S/\sqrt{n}}$$

where the variance of z is:

$$S^2 = \frac{\Sigma z^2 - \frac{(\Sigma z)^2}{n}}{n - 1}$$ (Campbell, 1967)

Therefore, $S^2 = 1.62$ and $S = 1.27$

$t_{obs} = 4.96$

Critical t for 95% confidence and 18 degrees of freedom (two-tailed test) = 2.101

Decision: Observed t is greater than the critical t and the null hypothesis is rejected. There is a significant difference between the mean root weight of control and infested plants.

Data which showed no significant linear correlation of differences (control minus infested) and whose mean populations (control and infested) were not significantly different as determined by the paired t-test, were considered identical (i.e., any variations occurring were due to random variations).

4-10. D. Analysis of Variance

This statistical analysis was used exclusively for data relating extent of root galling to gas concentrations. Plants were grouped in four columns according to gall severity (see methods) and a one-way analysis of variance computed. The test compared means across groups and determined how much of the difference was due to population differences and how much to random variability. A computed F-value was used to determine the level of significance of the differences. The null hypothesis can be written as

H_0: $u_1 = u_2 = \ldots = u_k$

and the F-test of this hypothesis as

$$F = \frac{\text{sum of squares between groups}/k - 1}{\text{sum of squares within groups}/n - k}$$

$$= \frac{n - k \sum_{i=1}^{k} \frac{1}{n_i} \left(\sum_{j=1}^{n_i} x_i \right)^2 - \frac{1}{n} \left(\sum_{i=1}^{k} \sum_{j=1}^{n_i} x_{ij} \right)^2}{k - 1 \sum_{i=1}^{k} \sum_{j=1}^{n_i} x_{ij}^2 - \sum_{i=1}^{k} \frac{1}{n_i} \left(\sum_{j=1}^{n_i} x_{ij} \right)^2}$$

which has $k - 1$ and $n - k$ degrees of freedom,

and, n_1 to n_k = size of independent random samples

k = number of populations from which n taken

x_{ij} = j-th observation of the i-th population.

(Wang Laboratories Inc., 1973)

EXAMPLE 7. Experiment 5. Ethylene versus Extent of Galling.

	Percentage Estimate of Root Galled			
	Group 1 (0%)	Group 2 (1-24%)	Group 3 (25-49%)	Group 4 (50-100%)
Ethylene Percentage Differences (Control and Infested)	6, 15, 94[1] -14, 16, -12	-204, 16, -114 -27, -32, -110	49, 52, -38 -2, 47, -49 -50, -12, -2 -23, -61, -15	33, 17 -24, 3
Mean Percentage Differences	17.5	-78.5	-8.7	7.3

[1] Positive values indicate control greater than infested, negative the opposite

Source	Sum of Squares	Degrees of Freedom	Mean Square
Between groups	32976.83	3	10992.28
Within Groups	58740.42	24	2447.52
Total	91717.25	27	

F-value = 4.49

Critical F-value for 95% confidence with 3 and 24 degrees of freedom = 3.01

Decision: The null hypothesis is rejected. The means of the groups are different within 95% confidence.

A Wang 2200 computer system was used for all one-way analysis of variance computations. Where taped programs were available, this system was utilized including mean, standard deviation and variance analyses, linear regressions without a zero intercept and paired t-test analysis.

5. RESULTS

Results are presented in this section on an experiment-by-experiment basis. It was necessary to interpret the data this way since some experimental conditions were altered between experiments. Changes in the age of plants used in each experiment, the season in which plants were grown, number of infectious nematode larvae applied and whether the plants were susceptible or resistant made it necessary to analyze data from each experiment individually. The conditions for each experiment are summarized in the following table. Each experiment was designed so that results obtained were extensive enough to be analyzed by statistical techniques. Where statistical significance was found, it refers to the 95% level of confidence. Marginal significance refers to a 90% level of confidence and in cases where great significance in the result was obtained, it is indicated in brackets (i.e., 99%).

Table 2. Summary of Experimental Conditions for Experiments 1 to 6.

Experiment	Plant Phenotype	Date Conducted	Age of Plants	Numbers of Nematodes Applied
1	susceptible var. Vendor	Jan-Feb 1977	46 days old at infestation	2,000 per plant
2	susceptible var. Vendor	May-June 1977	95 days old at infestation	10,000 per plant
3	susceptible var. Vendor	July-Aug 1977	90 days old at infestation	18,000 per plant
4	resistant var. Anahu	July-Aug 1977 run concurrent with experiment 3	90 days old at infestation	18,000 per plant
5	susceptible var. Vendor	Dec-Jan 1977-78	125 days old at infestation	17,000 per plant
6	resistant var. Anahu	Dec-Jan 1977-78 run concurrent with experiment 5	125 days old at infestation	17,000 per plant

5-1. Growth of Plants

Experiment 1. Susceptible Tomato Plants

Stem Heights

Stem heights of both control and nematode infested plants increased in a linear fashion for the duration of experiment 1 (Fig. 8A). At day 2 these were about 6.5 cm and they increased to 24.7 cm and 21.8 cm by day 43 for control and infested plants respectively. The graphs indicate no noticeable differences between control and infested stem heights up to day 18, however from this point on there is a tendency for infested stem heights to be less than controls. The differences (control minus infested) were analyzed statistically to determine if there was a linear correlation between increasing or decreasing differences with time. The linear regression did not satisfactorily fit the data. It was assumed then that overall the differences in stem height were distributed about a mean value. A paired t-test was used to compare the mean of one set to the mean of the other and determine if they were significantly different. Results showed no significant difference existed at the 95% level of significance, however there was a difference at the 80% level, indicating that the trend to difference from day 18 on may have been due to nematode infestation.

Top Weights

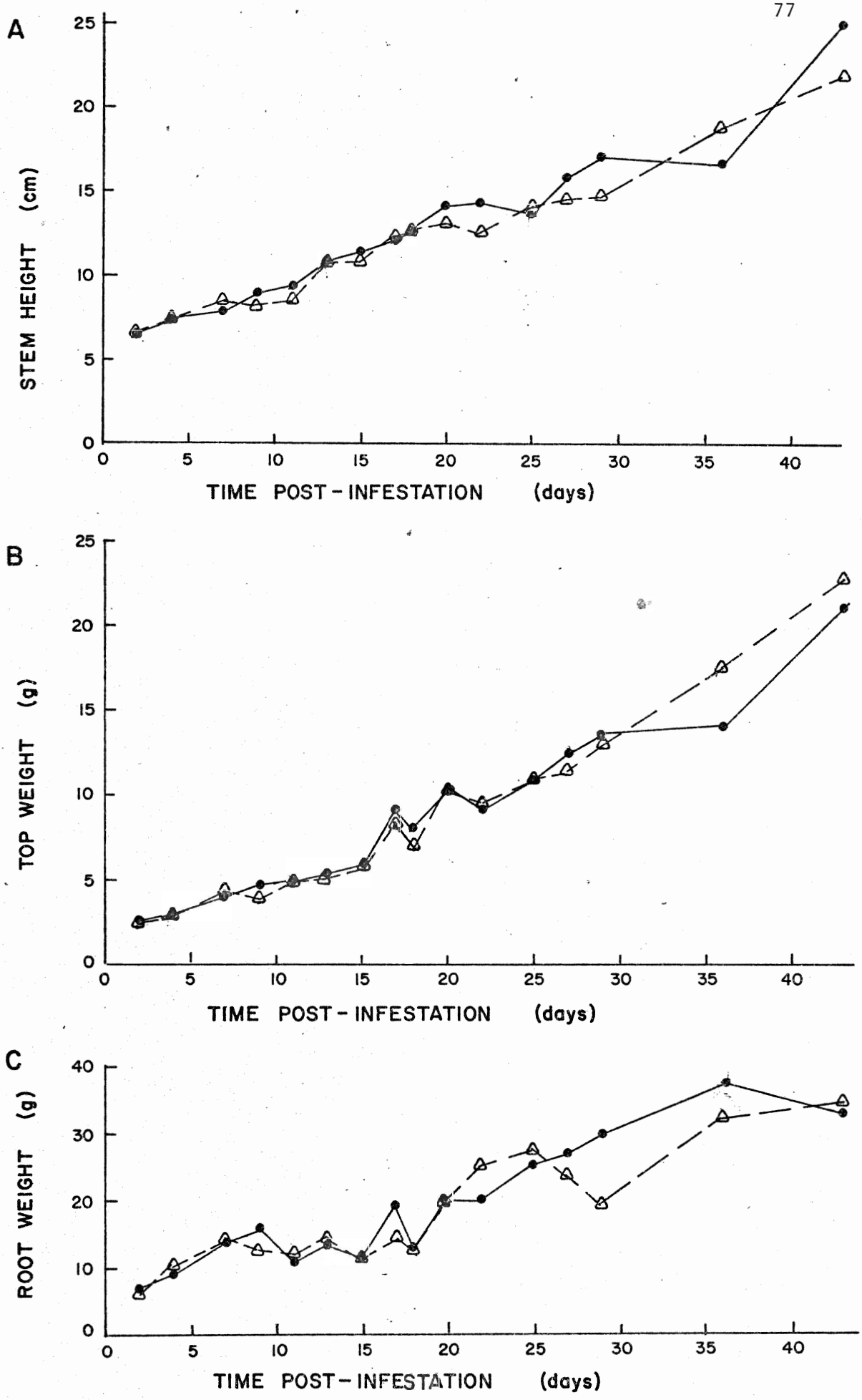
Beginning at 2.5 grams per plant, top weights increased in a nearly linear manner to levels of 21.0 and 22.6 grams by day 43 for control and infested plants respectively (Fig. 8B). Analysis of the differences revealed a linear correlation of increasing (C - I) differences with time. Top weights of infested plants were clearly greater than their controls by days 36 and 43 post-infestation. Paired t-test analysis, however,

Figure 8A-C. Growth measurements of the susceptible plants of Experiment 1. Each point on all graphs is the mean value from 8 plants (● control, Δ infested).

A. Graph of stem heights versus time post-infestation.

B. Graph of top weights versus time post-infestation.

C. Graph of root weights versus time post-infestation.


A
STEM HEIGHT (cm)
0
5
10
15
20
25
0
5
10
15
20
25
30
35
40
TIME POST-INFESTATION (days)
B
TOP WEIGHT (g)
0
5
10
15
20
25
0
5
10
15
20
25
30
35
40
TIME POST-INFESTATION (days)
C
ROOT WEIGHT (g)
0
10
20
30
40
0
5
10
15
20
25
30
35
40
TIME POST-INFESTATION (days)

determined that the mean top weights of control and infested plants were not significantly different.

Root Weights

Figure 8C reveals that root weights increased over the course of the experiment from values of approximately 2.8 grams to 13.4 grams for both control and infested plants. No obvious differences are apparent in the graphs and both linear regression analysis with time and the paired t-test indicated there were no significant differences in root weights of control and infested plants. Galls were observed on only a small percentage of roots of these infested plants.

Experiment 2. Susceptible Tomato Plants

Stem Heights

Figure 9A illustrates the generally linear increase of stem heights from about 25 cm to 70 cm and 55 cm for control and invested plants respectively. There was no apparent consistant difference in these up to day 22, however after this time there was a marked trend to greater stem heights of control plants. Linear regression analysis of the differences versus time fits significantly (99%), indicating that less stem elongation was occurring with time in infested plants.

Top Weights

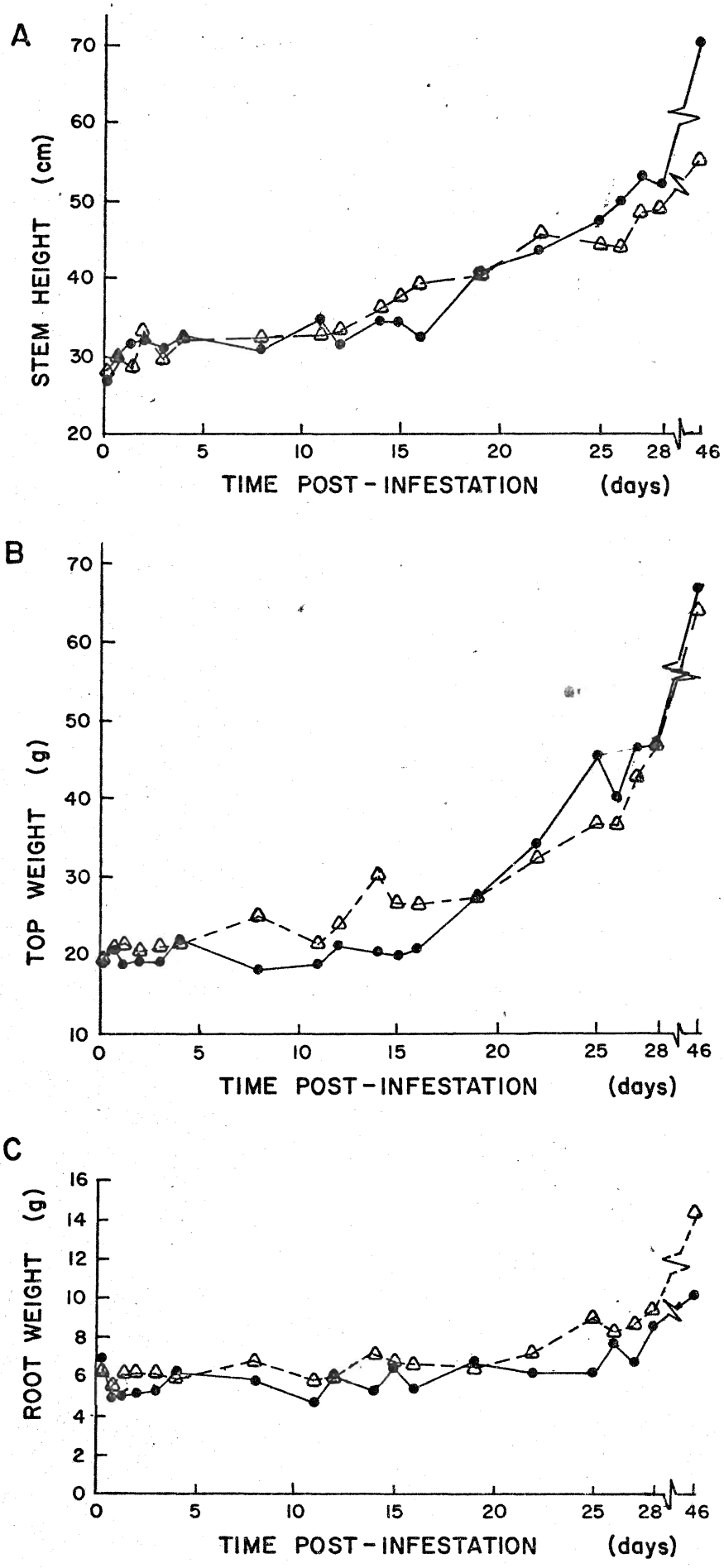
Plants increased in top weight from about 40 grams to 130 grams in the course of experiment 2 (Fig. 9E). Linear regression and paired t-test

Figure 9A-C. Growth measurements of the susceptible plants of Experiment 2. Each point on all graphs is the mean value from 4 plants (● control, Δ infested).

A. Graph of stem heights versus time post-infestation.

B. Graph of top weights versus time post-infestation.

C. Graph of root weights versus time post-infestation.


A
STEM HEIGHT (cm)
70
60
50
40
30
20
0
5
10
15
20
25
28
46
TIME POST-INFESTATION (days)
B
TOP WEIGHT (g)
70
60
50
40
30
20
10
0
5
10
15
20
25
28
46
TIME POST-INFESTATION (days)
C
ROOT WEIGHT (g)
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
28
46
TIME POST-INFESTATION (days)

analysis revealed that there was no significant difference between control and infested plants.

Root Weights

Root weights in experiment 2 increased from 12 grams to 20.5 grams and 28.5 grams for control and infested plants respectively (Fig. 9C). Infested roots were heavier than controls almost from the time of infestation with the difference being most consistent and marked from day 22 onwards. Linear regression analysis revealed that there was a significant linear correlation between increasing differences and time (99%). Considerable galling progressed with time in infested roots (see Figs. 37, 38 plate 4) which accounted for the heavier nematode-infested root weights.

Experiment 3. Susceptible Tomato Plants

Stem Heights

Plants of experiment 3 increased in stem height over the course of the experiment only slightly. Figure 10A shows that from day 4 onwards, control plants tended to elongate at a greater rate than infested. A linear increase of control with respect to infested stem heights over time was not significant. A paired t-test however indicated the mean control stem height to be greater than the mean infested stem height at the marginal level of significance.

Top Weights

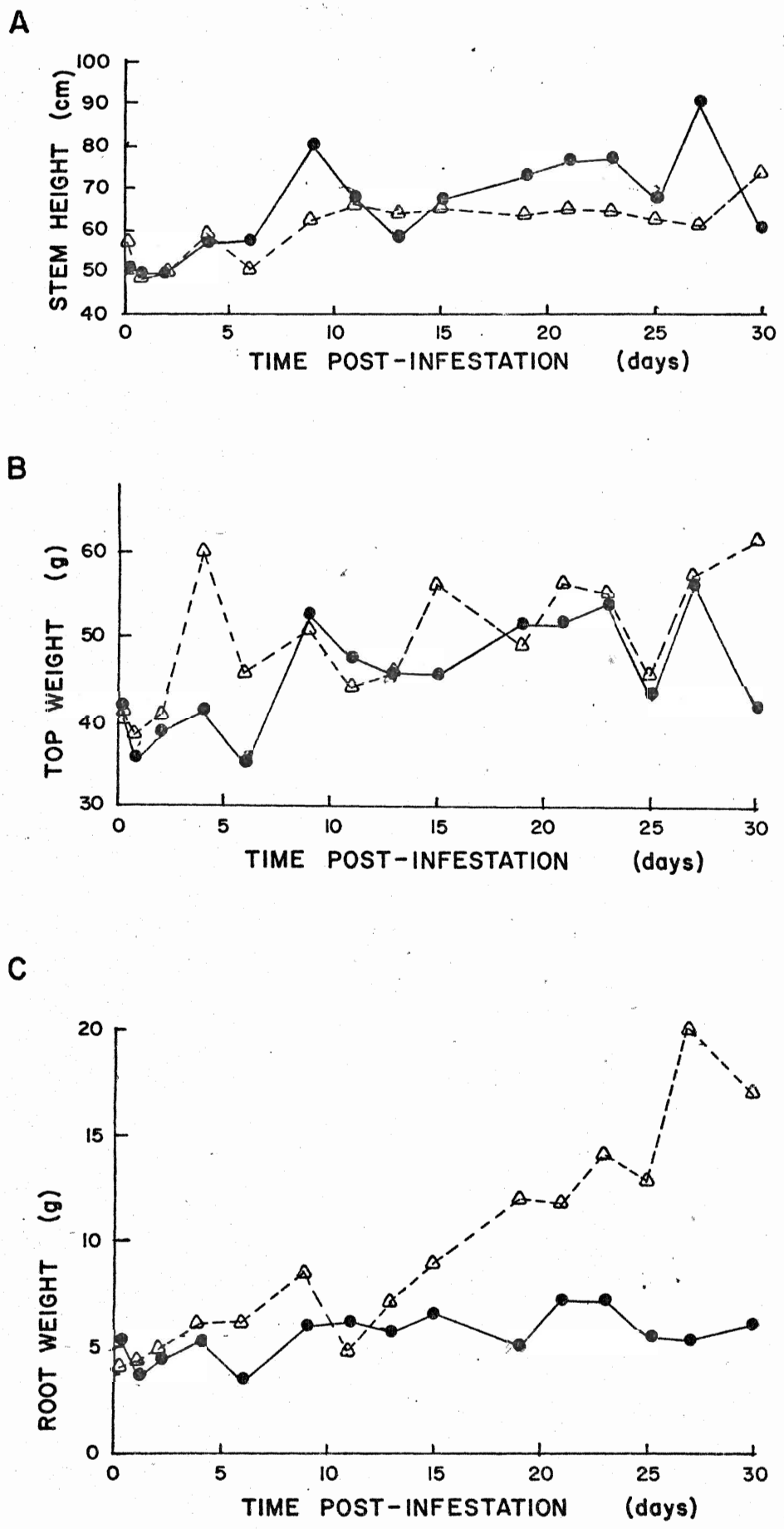
Top weights increased over the 30 days of experiment 3 from levels of about 40 grams each to approximately 50 grams control and 60 grams infested (Fig. 10B). Linear trends to increasing or decreasing differences between the data points were not apparent and linear regression analysis was negative

Figure 10A-C. Growth measurements of the susceptible plants of Experiment 3. Each point on all graphs is the mean value from 2 plants. (● control, Δ infested)

A. Graph of stem heights versus time post-infestation.

B. Graph of top weights versus time post-infestation.

C. Graph of root weights versus time post-infestation.


A
STEM HEIGHT (cm)
100
90
80
70
60
50
40
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)
B
TOP WEIGHT (g)
60
50
40
30
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)
C
ROOT WEIGHT (g)
20
15
10
5
0
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)

in this respect. Values for infested plants appear from the graphs to be greater than their control counterparts and a paired t-test analysis confirmed this observation.

Root Weights

Figure 10C shows a slight increase in control root weight with time while infested roots increased dramatically reaching 3 times the weight of controls by day 30. This trend was noticeable by day 4 and was obvious from day 13 onwards. Linear regression analysis of the difference between control and infested root weights with time was highly significant (99.9%). Galling of infested roots was observed to proceed rapidly and extensively so that roots were severely galled by the end of the experiment (see Figs. 37, 38 plate 5).

Experiment 4. Resistant Tomato Plants

Experiment 4 was run concurrently with experiment 3 so that the plants from these experiments were subjected to the same growth conditions. They also were of the same age at the start of the experiment and were treated identically as those of experiment 3 with nematodes.

Stem Heights

As in experiment 3, plants of experiment 4 increased slightly in stem heights with time which indicated that the plants were growing at a slow rate. Stem heights of control and infested plants were similar up to day 15, after which time those of infested plants were regularly greater than controls (Fig. 11A). Linear regression analysis showed no significant

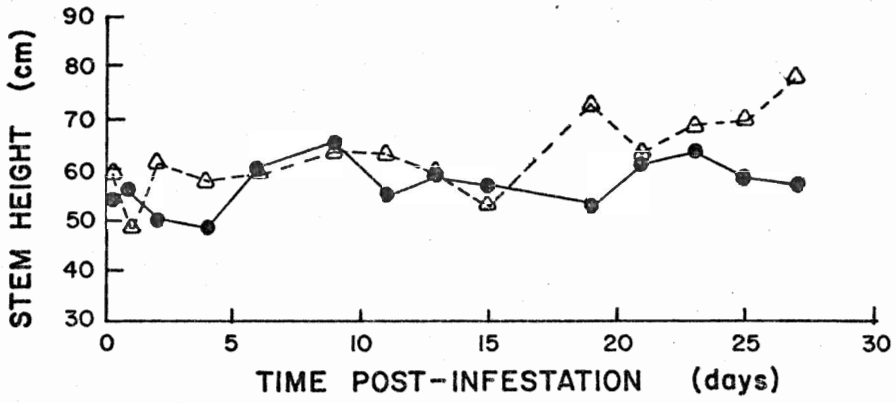
Figure 11A-C. Growth measurements of the resistant plants of experiment 4. Each point of all graphs is the mean value measured from a total of 2 plants. (● control, Δ infested).

A. Graph of stem heights versus time post-infestation.

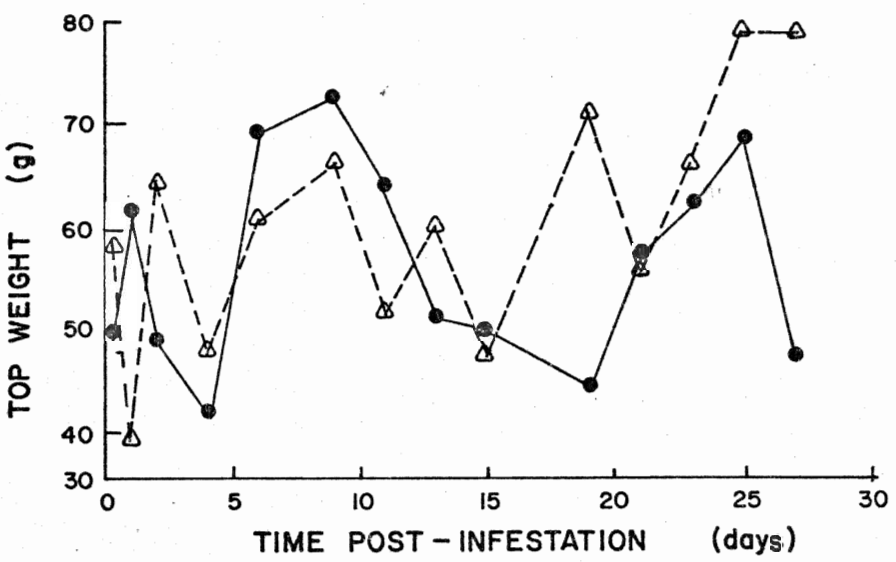
B. Graph of top weights versus time post-infestation.

C. Graph of root weights versus time post-infestation.

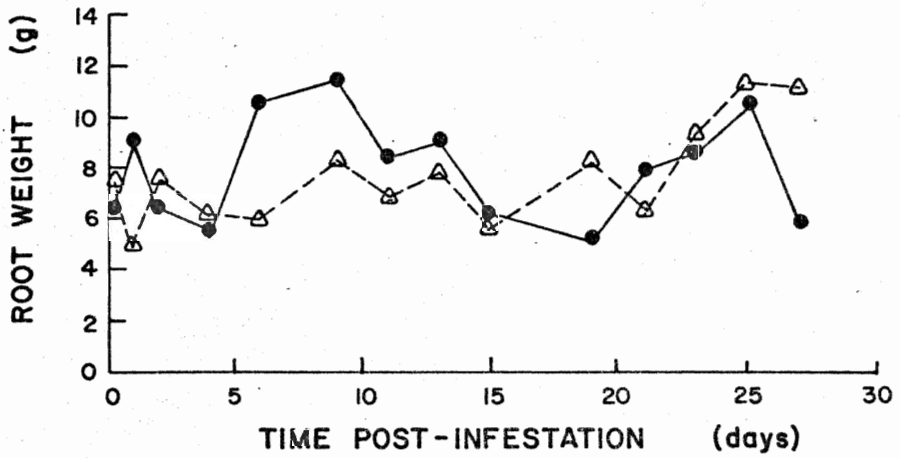
A


STEM HEIGHT (cm)
90
80
70
60
50
40
30
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)

B


TOP WEIGHT (g)
80
70
60
50
40
30
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)

C


ROOT WEIGHT (g)
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)

correlation between differences in height with time. Paired t-test analysis determined that the mean infested stem height was significantly greater than the mean control stem height.

Top Weights

Variations in the data on top weights of plants are immediately apparent from Figure 11B. Both linear regression and paired t-test analysis revealed no significant differences between top weights of control and nematode-treated plants.

Root Weights

Figure 11C indicates some root growth occurred for all plants during experiment 4. Consistent differences between control and infested plants are lacking. Both linear regression and paired t-test analysis confirmed that no significant difference existed. As well, no galls were ever observed on nematode-treated roots of experiment 4 plants.

Experiment 5. Susceptible Tomato Plants

Stem Heights

Plants of experiment 5 showed no growth over the course of the experiment as determined by measurements of stem heights, these being mostly in the range of 60-70 cm (Fig. 12A). Infested stem heights were consistently greater than their controls over the course of the experiment. The difference between the two sets of data was quite uniform and the absence of any significant correlation with time substantiated this observation. The mean infested stem height was determined to be significantly greater than the mean control stem height by paired t-test analysis.

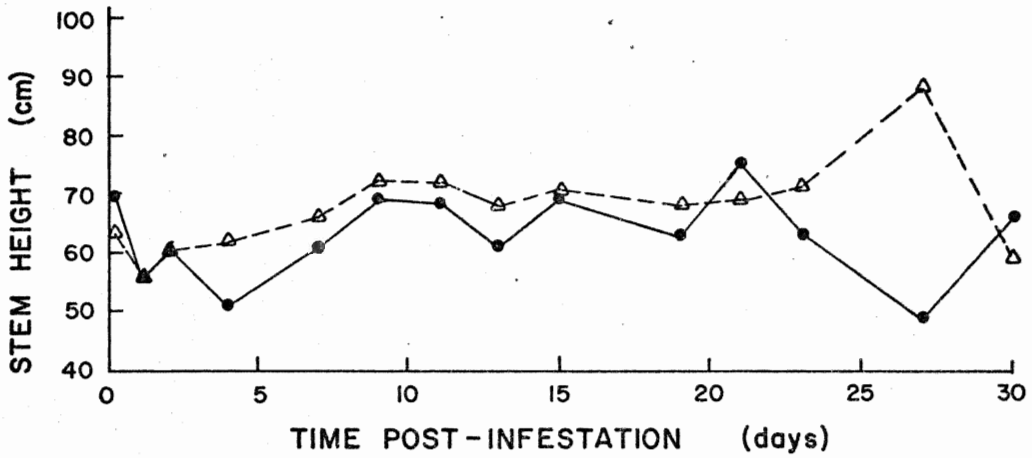
Figure 12A-C. Growth measurements of the susceptible plants of experiment 5. Each point on all graphs is the mean value measured from a total of 2 plants. (● control, Δ infested).

A. Graph of stem heights versus time post-infestation.

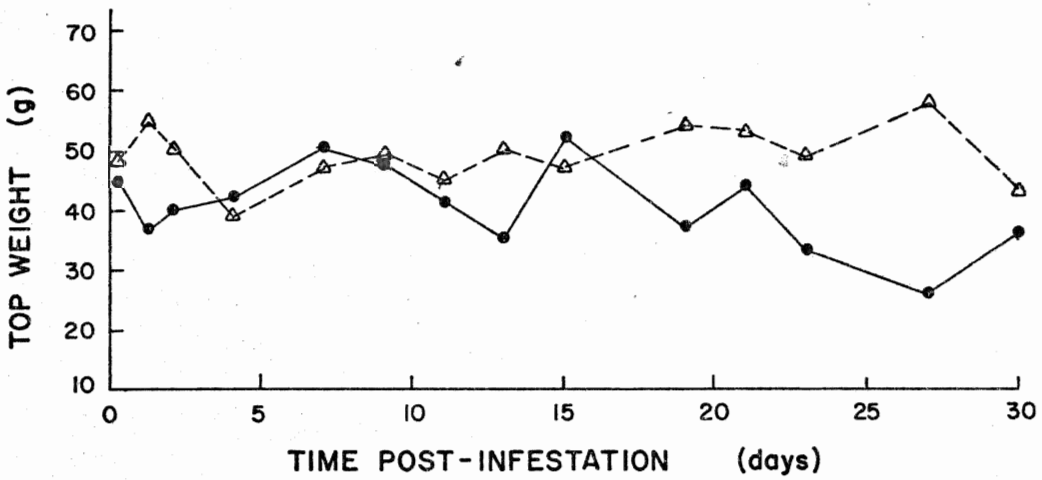
B. Graph of top weights versus time post-infestation.

C. Graph of root weights versus time post-infestation.

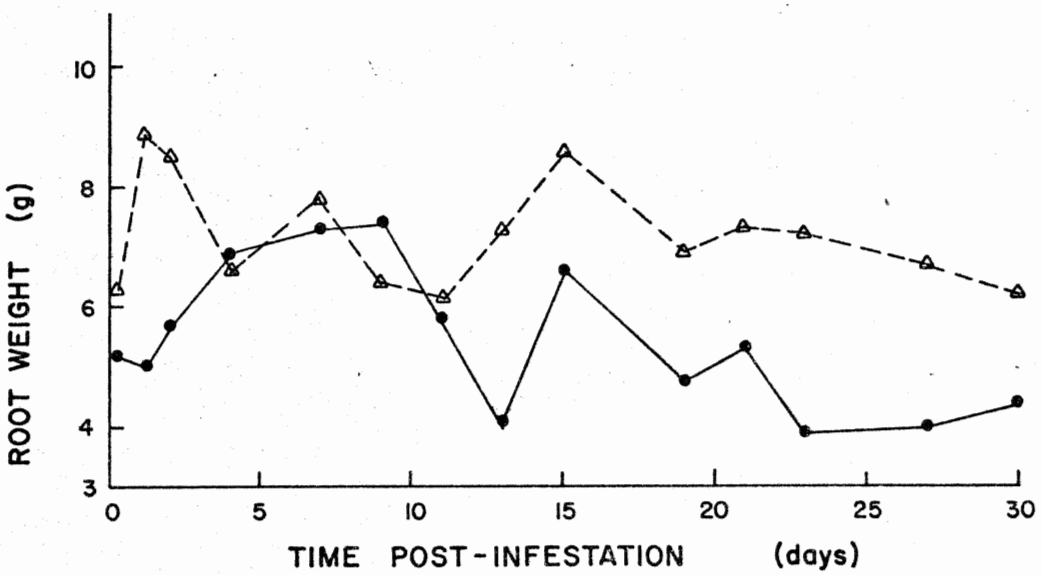
A


STEM HEIGHT (cm)
100
90
80
70
60
50
40
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)

B


TOP WEIGHT (g)
70
60
50
40
30
20
10
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)

C


ROOT WEIGHT (g)
10
8
6
4
3
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)

Top Weights

Top weights did not increase for either control or infested plants in experiment 5 (Fig. 12B). Infested plants remained about 50 grams per plant throughout the 30 experimental days. Control plant top weights decreased in this same time span as a result of senescence and abscission of leaves. Infested top weights were regularly larger than controls. Linear regression analysis of the differences with time showed no significant correlation, however the mean infested top weight was significantly greater than the mean control top weight as determined by paired t-test analysis.

Root Weights

Figure 12C demonstrates that infested roots did not appear to change in weight during the experiment but were largely constant at about 7.0 grams per root. In contrast control roots decreased in weight over the 30 day duration. Linear correlation of changing differences in root weights with time were not significant. Paired t-test analysis determined the mean infested root weight was significantly greater than the mean control root weight. Galling advanced at a moderate rate in infested roots (see Figs. 37, 38 plate 3).

Experiment 6. Resistant Tomato Plants

Experiment 6 was conducted concurrently with experiment 5 so that the plants of both experiments were all 125 days old at nematode-treatment and were subject to the same experimental conditions.

Stem Heights

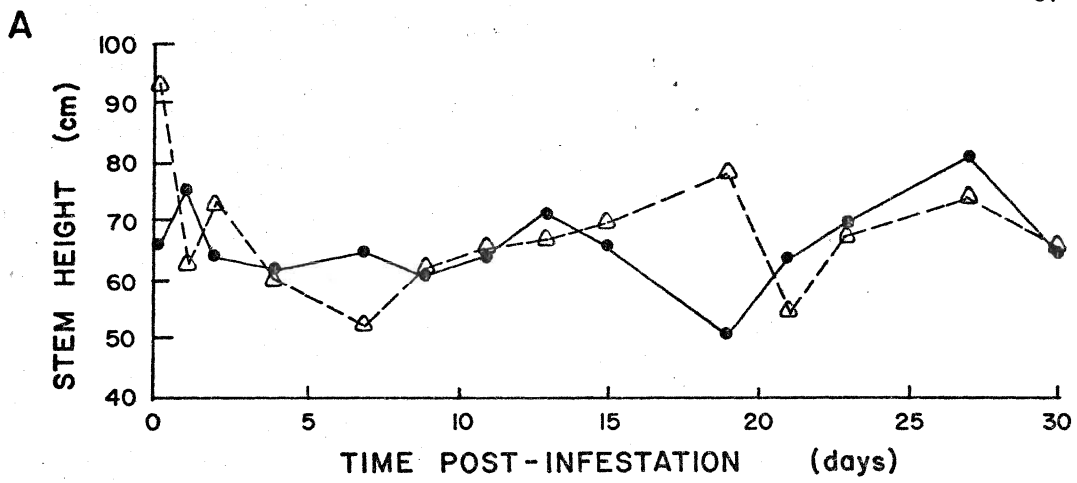
Figure 13A indicates that stems of both control and nematode-treated

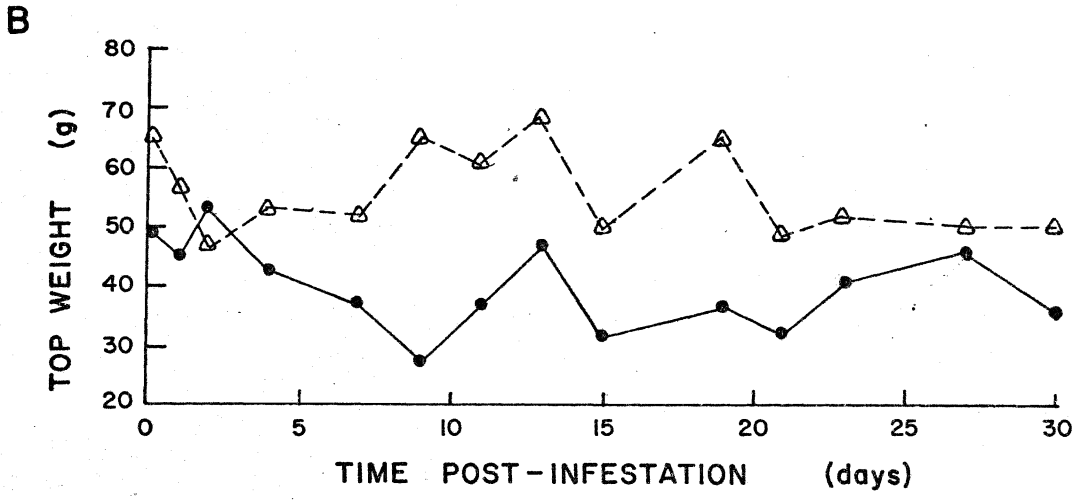
Figure 13A-C. Growth measurements of the resistant plants of experiment 6. Each point of all graphs is the mean value measured from a total of 2 plants. (● control, Δ infested).

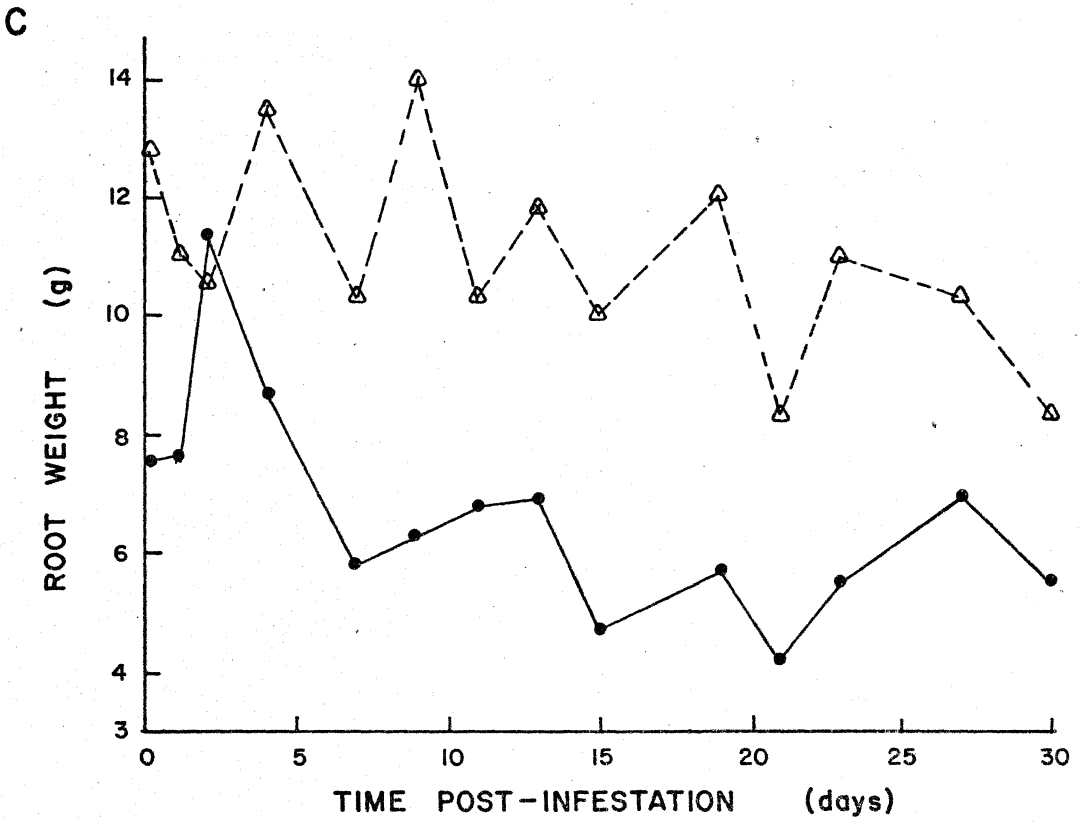
A. Graph of stem heights versus time post-infestation.

B. Graph of top weights versus time post-infestation.

C. Graph of root weights versus time post-infestation.


A
STEM HEIGHT (cm)
100
90
80
70
60
50
40
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)


B
TOP WEIGHT (g)
80
70
60
50
40
30
20
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)


C
ROOT WEIGHT (g)
14
12
10
8
6
4
3
0
5
10
15
20
25
30
TIME POST-INFESTATION (days)

plants did not increase in length over the 30 days. With respect to each other, no consistent differences between control and nematode-treated plants were found by linear regression and paired t-test analyses.

Top Weights

Plants treated with nematodes had top weights constantly greater than controls from day 4 onwards (Fig. 13B). The differences did not yield a significant linear correlation with time. Mean nematode-treated weight was determined to be significantly greater than mean control top weight. Top weights of both control and nematode-treated plants did not increase over the 30 days indicating little or no active plant growth was occurring.

Root Weights

Root weights of both control and nematode-treated plants decreased somewhat throughout the 30 day experiment. A large, fairly uniform difference between the lines of figure 13C is obvious with nematode-treated roots being heavier. This difference did not change significantly with time as determined by a linear regression. Paired t-test analysis determined the mean nematode treated root weight to be significantly greater than the mean control root weight.

A summary of root-knot nematode effects on growth data is presented in Table 3. In 3 out of 4 susceptible experiments, infested root weights and infested top weights were greater than controls. A tendency for control stem heights to be less than infested stem heights was observed in 2 of the 4 susceptible experiments.

Table 3. Summary of Meloidogyne incognita effects on growth of susceptible and resistant plants.

Experiment and Date	Physiological Growth Stage of Experimental Plants	Statistical Analysis of Growth Data		
		Stem Heights	Top Weights	Root Weights
Exp. 1 Susceptible Jan/Feb 1977	46 days old at infestation young--rapidly growing	NS	I > C (LR)	NS
Exp. 2 Susceptible May/June 1977	95 days old at infestation maturing plants--growing but less rapidly as Exp. 1 plants	C > I (LR)	NS	I > C (LR)
Exp. 3 Susceptible July/Aug 1977	90 days old at infestation mature plants--slow growth less than plants of Exp. 2	C > I (tT)*	I > C (tT)	I > C (LR)
Exp. 5 Susceptible Dec/Jan 1977-78	125 days old at infestation stable physiological age--no measurable active growth	NS	I > C (tT)	I > C (tT)
Exp. 4 Resistant July/Aug 1977	90 days old at infestation mature plants--slow growth less than plants of Exp. 2	N.T. > C (tT)	NS	NS
Exp. 6 Resistant Dec/Jan 1977-78	125 days old at infestation stable physiological age--no measurable active growth	NS	N.T. > C (tT)	N.T. > C (tT)

I = infested, C = control plants, N.T. = Nematode-treated resistant plants
NS = no significant difference between control and infested
I > C, C >I etc. = a significant difference (95%) as indicated
* = marginal significance (90%) only
(LR) = significance determined by linear regression analysis
(tT) = significance determined by t-test analysis

5-2. Time Course of Gas Extraction from Roots

Results of experiments designed to establish the volume of gas collected with time exposed to evacuation are shown in Figure 14A. Resistant and susceptible tomato varieties exhibited the same pattern. Eighty percent of the gas collected over the 60 minutes of extraction was obtained in the first 10 minute interval for the seven experiments (susceptible 83%, resistant 76%). After 20 minutes extraction a mean of 85% of the total gas had been collected (susceptible 87%, resistant 81%). Only 5% more gas was obtained with the second 10 minute evacuation. It was further observed that gas bubbles emerged most rapidly and consistently in the first 10 minutes of the gas extractions in experiment 1 (gases were extracted for 45 minutes in experiment 1). It was therefore decided to extract gases for 15 minutes per run in experiment 2.

Two mercury manometers were installed on the gas extraction apparatus by run 15 of experiment 2. This was necessary since variations in water pressure were encountered from day to day and at different times of the day. Such variations could have affected the efficiency of the aspirator and hence the efficiency of gas extraction.

Results showing the drop in partial pressure of the gas extraction chamber with time of evacuation for run 15 of experiment 2 are shown in Figure 14B. An hyperbolic relationship existed whereby the drop in partial pressure was at first rapid but tapered off as it became less than 3.0 cm Hg. This pattern was consistent for both control and infested plants on either extraction apparatus. An end point of 2.0 cm Hg was reached with apparatus A after a mean extraction time of 11.5 minutes. Apparatus B, being slightly greater in volume, required a mean time of 15.5 minutes to reach the same partial pressure. These time intervals correlated closely with those

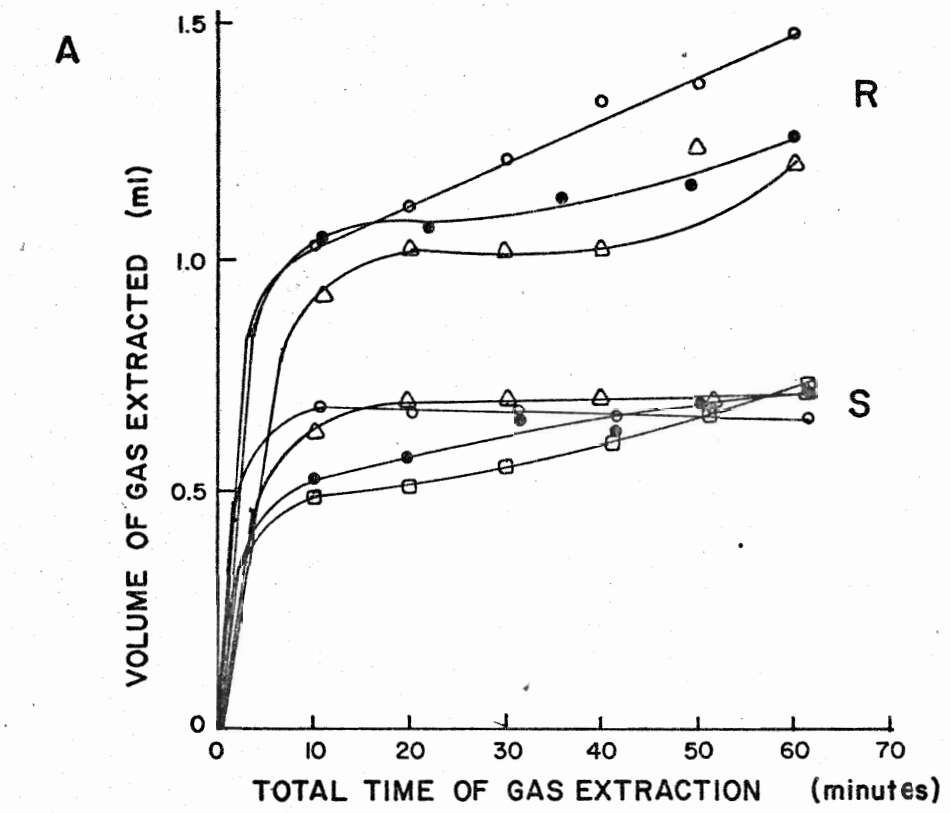
Figure 14A. The volume of gas extracted from tomato roots versus time of extraction.

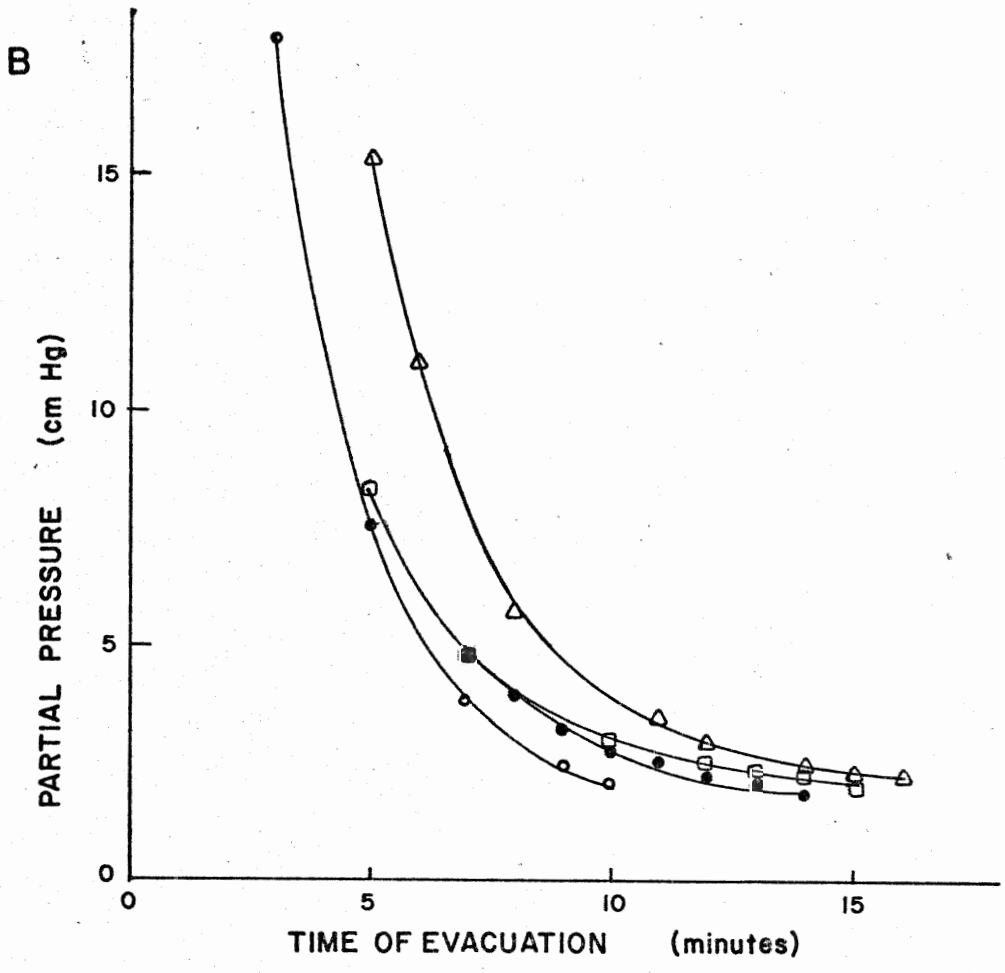
Gases were extracted from the roots of 4 plants per experiment. The total volume of gas extracted from the roots of each experiment was measured in 10 minute intervals over a total time of 60 minutes. Seven experiments were conducted, 4 on root-knot nematode susceptible plants (S) and 3 on resistant plants (R).

Figure 14B. Drop in partial pressure of the root gas extraction chamber versus time of aspiration.

The gas extractions of experimental run 15 of experiment 2 were monitored with mercury manometers attached to the evacuation chamber (Fig. 5). Two determinations for each control and infested roots were obtained. Two aspirators designated A and B were used for the determinations and in all subsequent experiments.

- • 15-1 control, aspirated A.
- □ 15-2 control, aspirator B.
- △ 15-1 infested, aspirator B.
- ○ 15-2 infested, aspirator A.


A
R
S
VOLUME OF GAS EXTRACTED (ml)
1.5
1.0
0.5
0
0
10
20
30
40
50
60
70
TOTAL TIME OF GAS EXTRACTION (minutes)


B
PARTIAL PRESSURE (cm Hg)
15
10
5
0
0
5
10
15
TIME OF EVACUATION (minutes)

determined to collect 85% of the total root gases. Since extraction of gases to a determined end point of partial pressure (2.0-3.0 cm Hg) should ensure more consistent results, this method was used for all subsequent experiments (i.e., experiments 3, 4, 5, 6).

5-3. Identification of Gases Extracted from Roots

A representative chromatographic trace of the gases extracted from tomato roots is shown in Figure 15A. Peaks 2 and 3 were consistently symmetrical as shown. Peak 1 was very sharp and always offscale. Peak 4 characteristically tailed off. Peak 5 usually was overrun by peak 1 but was sometimes distinct. Mean retention times calculated from numerous samples were: peak 1, 45 seconds; peak 2, 135 seconds; peak 3, 180 seconds and peak 4, 300 seconds.

Figure 15B is a typical tracing obtained from injection of an ethylene standard diluted with air. The ethylene peak obtained had a mean retention time of 135 seconds which coincided with the retention time of peak 2 in the sample gas tracing. A small sharp peak preceded the ethylene peak and had a mean retention time of 53 seconds. It was determined that injecting a sample of plain air or simply inserting the needle briefly into the injection port resulted in this same sharp peak (Fig. 15C, D). It was concluded that this small peak was an artifact resulting from a perturbation of gas flow in the column and was thereafter referred to as an air peak.

Sample gas tracings exhibited a much larger peak 1 than that obtained from air. Standard preparations of methane had a mean retention time of 45 seconds (Fig. 15E) and it was suspected that the off scale peak 1 was methane.

Standard preparations of ethane gave the characteristic trace seen in Figure 15F. Again the air peak was present and the ethane peak had a mean

retention time of 180 seconds which coincided with peak 3 of the sample gas tracings.

Root gases were shaken with mercuric perchlorate to absorb any olefins present and after flushing out unabsorbed gases were released with lithium chloride. The released gases resulted in tracings identical to those of ethylene standards. Peaks 1, 3 and 4 which were present in samples of the same root gases had disappeared leaving only peak 2 and the air peak. The mean retention time of peak 2 was 135 seconds. Standard ethylene preparations subjected to the same treatment produced identical tracings with a mean retention time of 135 seconds.

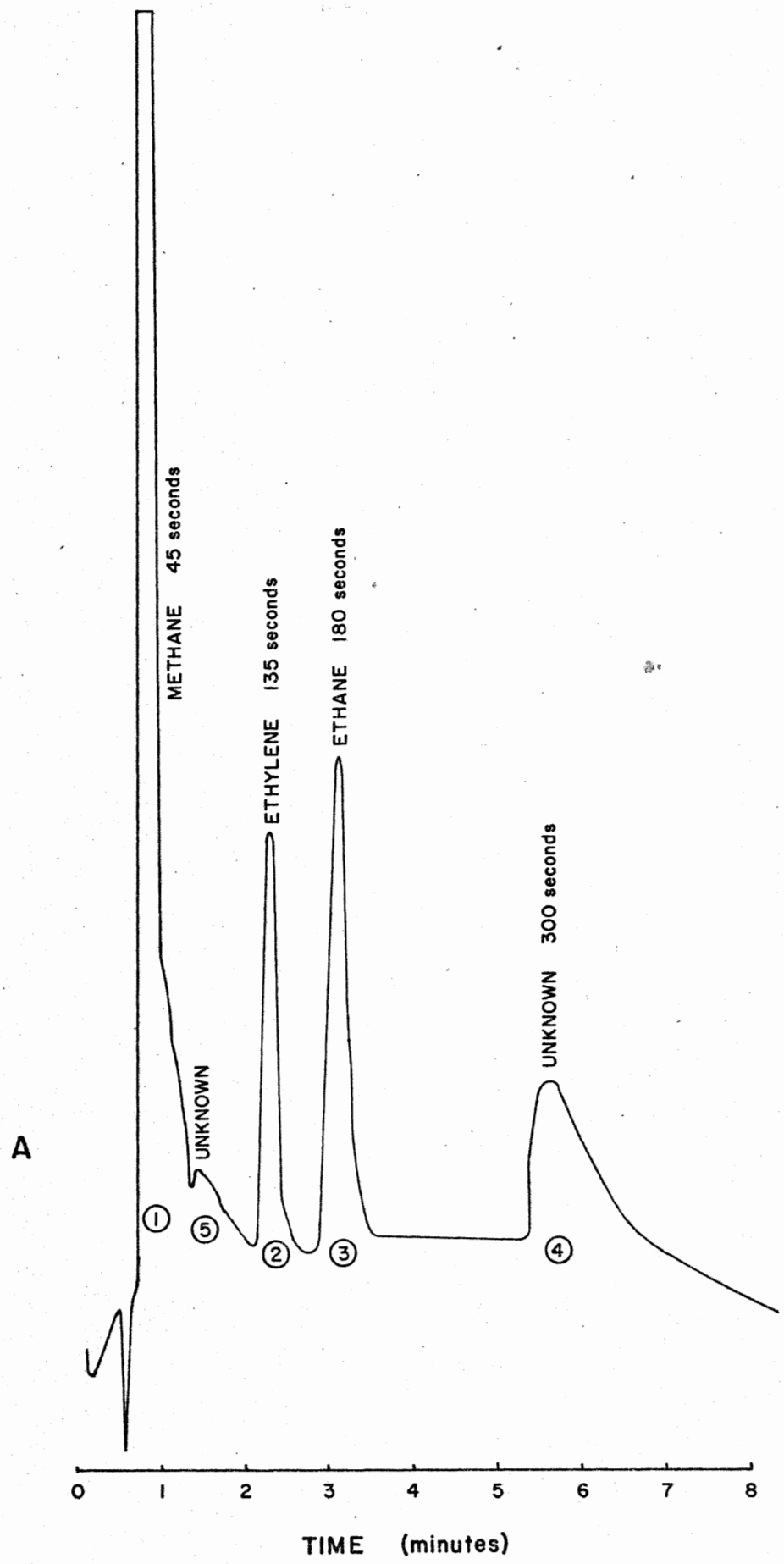
Unequivocal identification of the sample gas peaks was attempted with mass spectra data. A gas chromatograph-mass spectrophotometer (GC/MS) was used. The gas chromatograph had a thermal conductivity detector which was orders of magnitude less sensitive than the flame ionization detector used in experimental runs. Sample peaks were therefore undetectable on the gas chromatograph of the GC/MS and a direct analysis of the spectra accompanying sample gas peaks could not be obtained.

Methane and ethylene standards at higher concentrations than sample gases were analyzed with the GC/MS. The peaks obtained had retention times of 75 and 230 seconds and their corresponding mass spectra were characteristic of methane and ethylene respectively. A longer column (305 cm x .64 cm) accounted for the greater retention times. The ratio of methane retention time to ethylene retention time was however, identical from both gas chromatographs. Ethane standards were not analyzed. Although GC/MS experiments did not accomplish an unequivocal proof of the identity of sample gas peaks, the evidence supports the conclusion that peak 1 of the sample gases was methane and peak 2 ethylene.

Figure 15. Gas chromatographic traces of gases extracted from tomato roots (A) and of various gas standards (B-F).

Gases were analyzed on a Hewlett-Packard 5700A gas chromatograph equipped with a flame ionization detector. Retention times are those obtained with a carrier gas flow of 60 ml per minute.

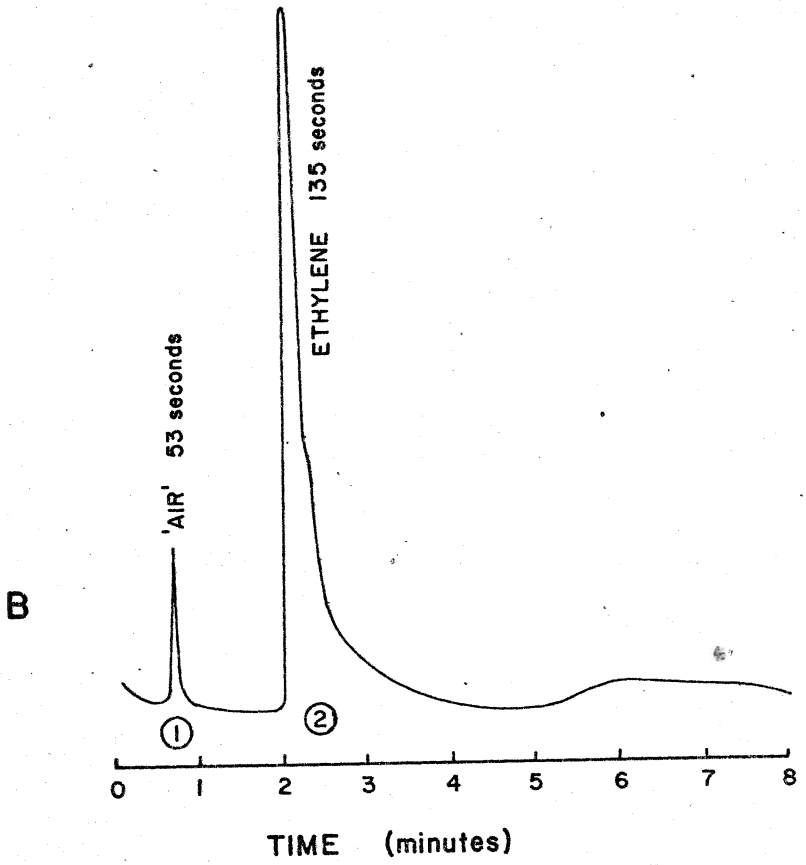
A. Tracing obtained on analysis of 1.0 ml sample of extracted root gas. Characteristic peaks are numbered, identified and their retention times indicated.

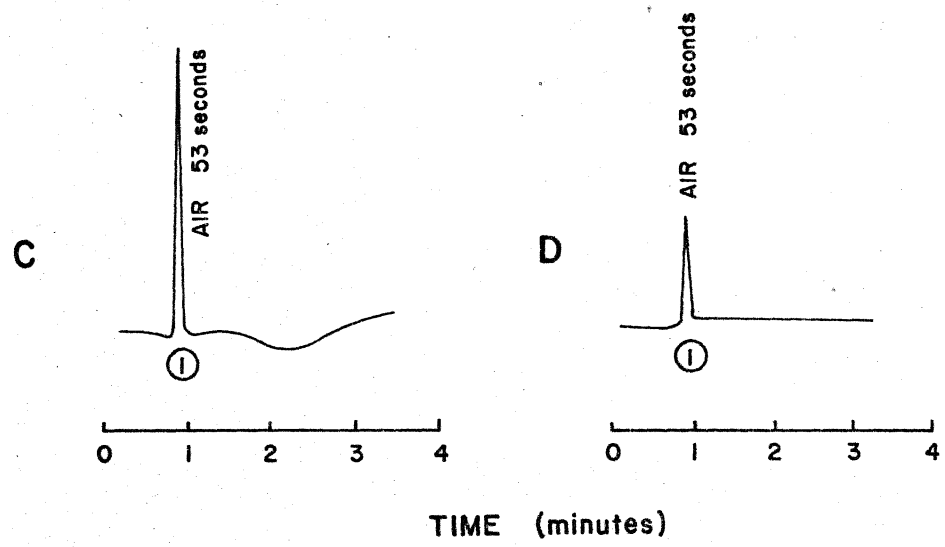

A
METHANE 45 seconds
UNKNOWN
ETHYLENE 135 seconds
ETHANE 180 seconds
UNKNOWN 300 seconds
1
5
2
3
4
0
1
2
3
4
5
6
7
8
TIME (minutes)

B. Tracing obtained on analysis of 0.1 ml of standard ethylene gas of concentration 65 µl/l. Only a small 'air' peak and the ethylene peak were obtained.

C. Tracing obtained on analysis of 1.0 ml sample of air.

D. Tracing obtained on insertion of hyperdermic needle into the injection port without deposition of any sample.

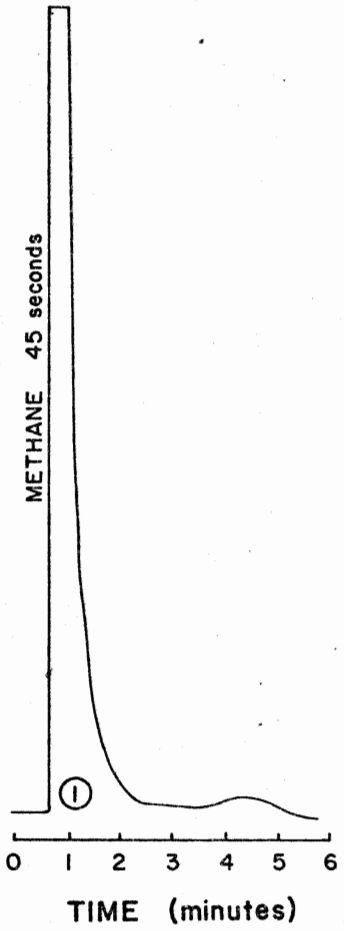

B
'AIR' 53 seconds
ETHYLENE 135 seconds
①
②
0 1 2 3 4 5 6 7 8
TIME (minutes)


C
AIR 53 seconds
①
0 1 2 3 4
D
AIR 53 seconds
①
0 1 2 3 4
TIME (minutes)

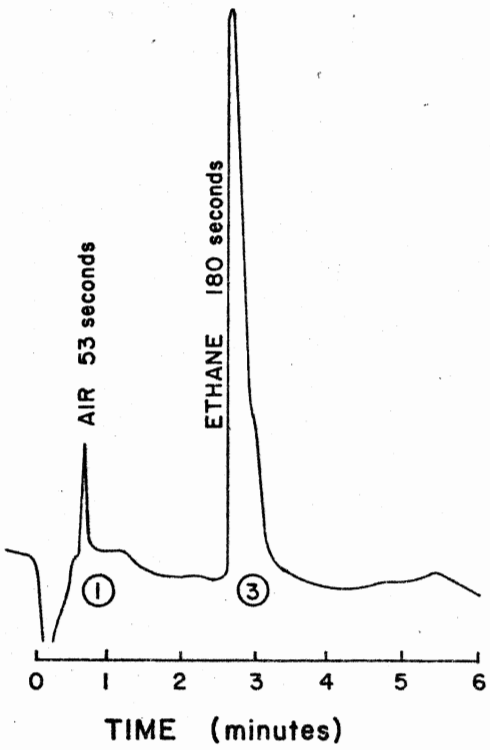
E. Tracing obtained on analysis of 0.01 ml of pure methane gas. The flat top of the peak indicates an offscale recorder response.

F. Tracing obtained of analysis of 1.0 ml of ethane standard gas of concentration 4.43 µl/l.

E


METHANE 45 seconds
1
0 1 2 3 4 5 6
TIME (minutes)

F


AIR 53 seconds
ETHANE 180 seconds
1
3
0 1 2 3 4 5 6
TIME (minutes)

Altered carrier gas flow rates (30 ml per minute) used in experiments 5 and 6 changed retention times of methane, ethylene and ethane to 80 seconds, 270 seconds and 315 seconds respectively.

5-4. Calibration Curves for Measuring Concentrations of Extracted Gases

Calibration curves indicating the size of peaks for known gas concentrations of ethylene and ethane were established. Peak areas were calculated by summing the counts recorded by the electronic integrator for each peak. Since experiments 1-4 were conducted under identical gas chromatographic conditions, data obtained with gas standards during this period have been pooled (appendix 2 tables 1-4) and a single calibration curve for ethylene and ethane computed (figs. 16, 17). All peak sizes obtained for each standard concentration were used in a linear regression analysis.

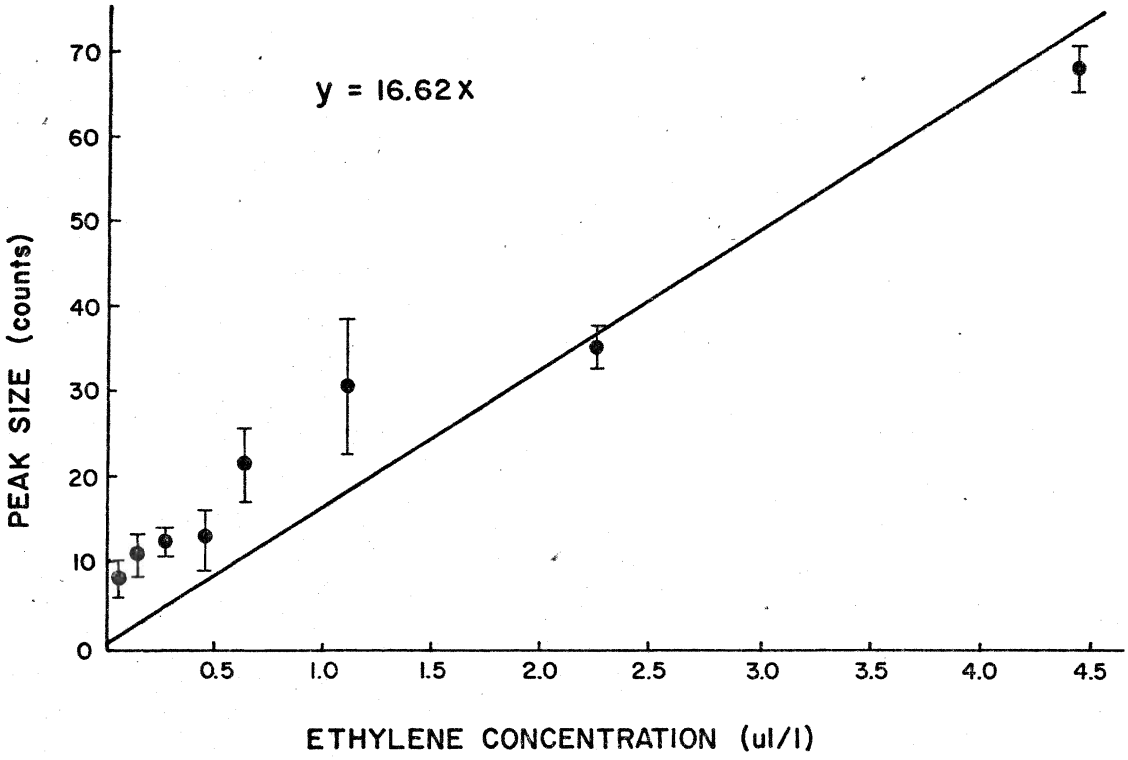
The analyses yielded the best straight line fit of the data as defined by the equation $y = \hat{\beta}_0 + \hat{\beta}_1 x$ (Mendenhall, 1975) where $\hat{\beta}_1$ is the slope and $\hat{\beta}_0$ the y-intercept. The best straight line to fit the data was in all cases highly significant (99%) which indicated that any daily variations of recorder sensitivity were within acceptable limits. Similarly the results of analysis of ethane and ethylene standards with the experimental conditions used in experiments 5 and 6 were pooled. The best straight line fit of these data as computed by linear regression analysis was significant at the 99.9% level.

Ethylene peak sizes in these experiments were generally in the range of standards used. In some runs of experiments 1-4, however, the ethylene peak size was less than the calculated y-intercept (9.63 counts) obtained from this analysis (appendix 2, tables 1-4). Since no ethylene peak was obtained

Figure 16. Calibration curves for ethylene determinations.

Calibration curves for ethylene were obtained from pooled data of individual sets of standards analyzed with each experiment. Gases for experiments 1-4 (A) were analyzed with a carrier gas flow rate of 60 ml nitrogen per minute while gases of experiments 5 and 6 (B) were analyzed with a nitrogen flow rate of 30 ml per minute. The lower rate of carrier gas flow resulted in a broadening of peak width and a concomitant shortening of peak height. This permitted standards of higher concentration to be analyzed with experiments 5 and 6 than was possible for experiments 1-4. Reproducibility of peak sizes with low gas concentrations was improved with the lower carrier gas flow rate of experiments 5 and 6.

A ETHYLENE STANDARD CURVE EXPERIMENTS 1-4



B ETHYLENE STANDARD CURVE EXPERIMENTS 5, 6

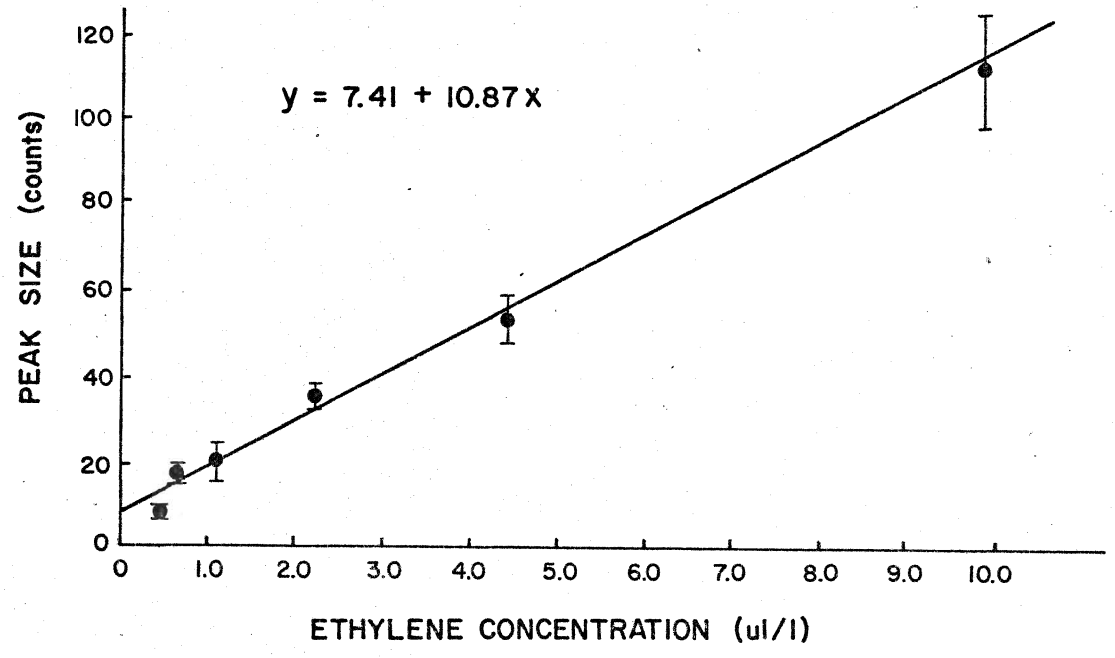
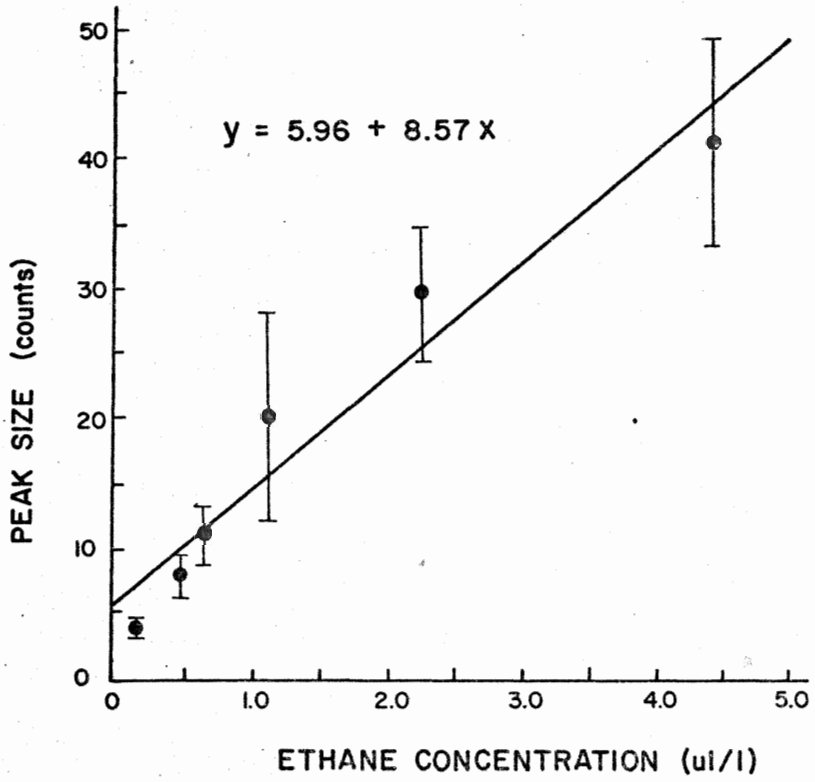


Figure 17. Calibration curves for ethane determinations.

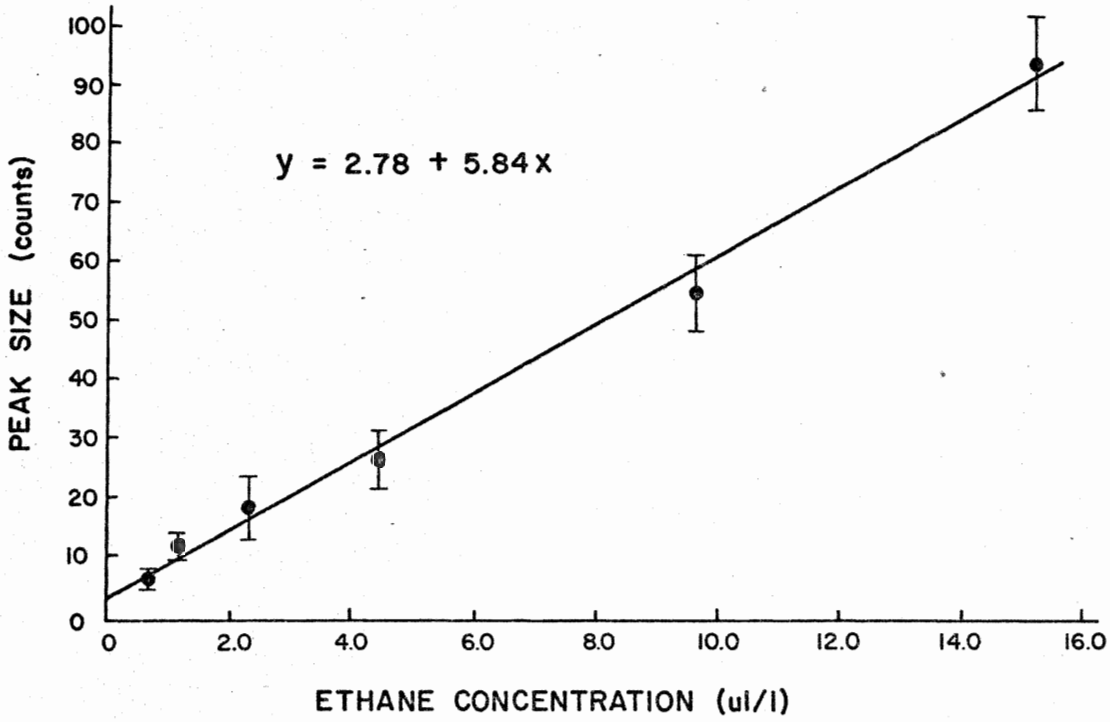
Ethane standard curves for experiments 1-4 (A) and 5-6 (B).

Nitrogen carrier gas flows were as described for ethylene curves.

A ETHANE STANDARD CURVE EXPERIMENTS 1-4



B ETHANE STANDARD CURVE EXPERIMENTS 5,6



with injection of air, the point (0,0) was included in the gas standard data. Exponential ($y = Ae^{Bx}$) and geometric ($y = Ax^{B}$) curves were fitted to the data but the most satisfactory fit was obtained with a straight line passing through the origin (statistical analysis example 5).

In all other experimental runs, peak sizes were within the range of standards selected and the line of best fit with a y-intercept was used. Statistically, this line fit the data to a higher degree of significance (99.9%) than when omitting the intercept (95%), however both were within acceptable limits of significance.

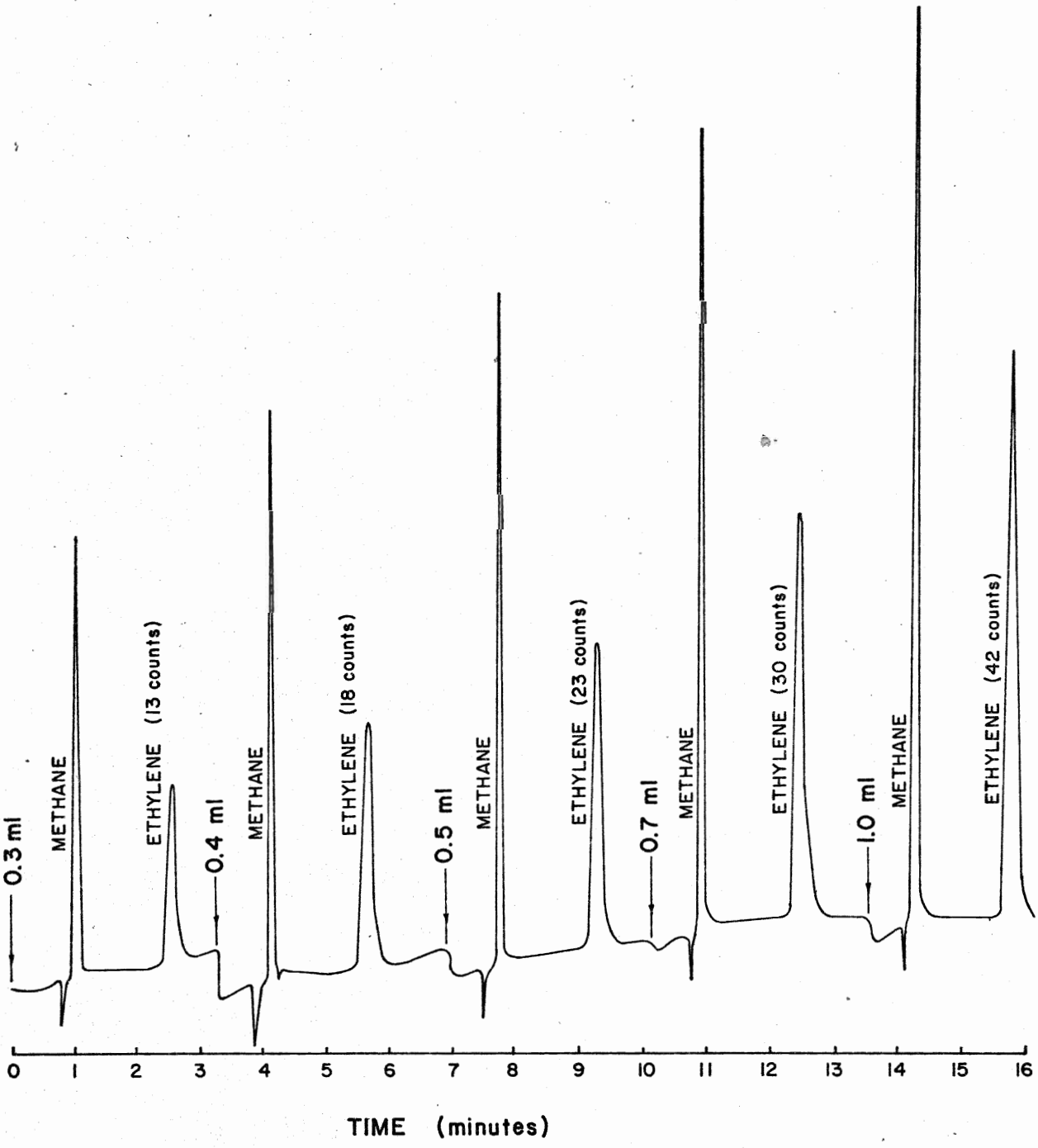
5-5. Calculation of Gas Concentrations

Areas under gas peaks were integrated electronically by a second pen on the chart recorder and peak size indicated by an arbitrary scale of counts. Experimental gas samples were usually less than 1.0 ml in volume. Standard ethylene and ethane preparations were analyzed in 1.0 ml aliquots and the calibration curves obtained correlated peak size (counts) to gas concentration (µl/l) for a 1.0 ml sample. Increasing or decreasing the volume of a sample of known concentration should result in proportional changes in peak size. Results of an experiment conducted to establish the truth of this assumption are illustrated in Figure 18. Increasing the amount of standard ethylene (2.34 µl/l) sample injected into the gas chromatograph resulted in a proportional increase in recorded peak size.

Ethane and ethylene peaks from experimental gas samples of less than 1.0 ml volume (approximately 90% of gas volumes analyzed were between 0.3 and 1.0 ml) were measured and then converted to the size appropriate for a 1.0 ml sample. For example, an ethylene peak size of 17.0 counts obtained

Figure 18. Relationship between gas volumes injected and the size of ethylene peaks.

Chromatographic tracings were obtained on injection of 0.3, 0.4, 0.5, 0.7 and 1.0 ml volumes of ethylene standard of concentration 2.32 ml/l. The standard preparation had a slight methane impurity. Both methane and ethylene peaks show a linear increase in size (counts) as the volume of gas injected was increased. Arrows indicate time of injection of gas sample.


0.3 ml
METHANE
ETHYLENE (13 counts)
0.4 ml
METHANE
ETHYLENE (18 counts)
0.5 ml
METHANE
ETHYLENE (23 counts)
0.7 ml
METHANE
ETHYLENE (30 counts)
1.0 ml
METHANE
ETHYLENE (42 counts)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
TIME (minutes)

from 0.60 ml sample gas was converted arithmetically to an equivalent 28.3 counts for a 1.0 ml sample. With the peak size expressed as that which would have been obtained from a 1.0 ml sample the concentration of the gas in microlitres per litre could be calculated from the standard curves by solving the equation of the line of best fit $y = \hat{\beta}_0 + \hat{\beta}_1 x$ where $\hat{\beta}_0$ = y-intercept (counts), $\hat{\beta}_1$ = slope of line (counts/µl/l), y = peak size (counts) and x = gas concentration (µl/l). Experiment 5 run 1-1 control had an ethylene peak size of 24.0 counts per 1.0 ml of sample gas. The equation of the line for the ethylene standard curve for experiments 5 and 6 (fig. 16B) was y = 7.41 counts + (10.89 counts/µl/l)(x). To solve this for x gas concentration, the appropriate value for y (peak size) was substituted in the rearranged equation

$$x = \frac{y - \hat{\beta}_0}{\hat{\beta}_1}$$

$$x = \frac{(24.0 \text{ counts} - 7.41 \text{ counts})}{(10.89 \text{ counts}/\mu l/l)} = 1.52 \ \mu l/l$$

All concentrations of experimental gas samples were calculated similarly using the appropriate standard curve.

In the final form gas concentrations were expressed as a weight of gas per gram root weight. Concentrations in µl/l were converted to a weight as follows. The weight of 1.0 ml of pure gas (ethane of ethylene) was calculated. The moles of gas in a given volume is dependent on temperature and pressure according to the relationship defined by the 'ideal gas law' PV = nRT where P = atmospheric pressure (ml Hg = torr), V = volume of gas (ml), n = number of moles of gas, R = universal gas constant (ml torr $deg^{-1}mole^{-1}$) T = temperature (degrees Kelvin) (Moore, 1972). The value of 1 mole of ethane or ethylene gas was calculated by rearranging this equation to

$V = nRT/P$. Mean temperature and pressure calculated from those recorded with each set of standards for a given calibration curve (data in appendix 2, tables 1-4) were substituted into this formula. Continuing with experiment 5 run 1-1c as an example,

constants, $n = 1$ mole,

$R = 0.62362 \times 10^5$ ml torr $deg^{-1}mole^{-1}$

experiment 5,6 ethylene standard curve mean values

$P = 755.3$ torr

$T = 295.1$ degrees Kelvin

Thus, $$V = \frac{(1 \text{ mole})(0.62362 \times 10^5 \text{ ml torr deg}^{-1}\text{mole}^{-1})(295.1 \text{ deg})}{755.3 \text{ torr}}$$

$V = 24{,}365$ ml

Therefore the volume of 1 mole of gas at the experimental conditions of experiments 5 and 6 was 24,365 ml. The number of moles of gas in 1.0 ml is the inverse of this or 4.10×10^{-5} moles. The gram molecular weight of ethylene is 28.06 grams per mole and multiplying this value with the number of moles in 1.0 ml gives the weight of pure ethylene in 1 ml, 1.15×10^{-3} grams. The weight of ethylene in 1.0 ml of root gas sample was found by multiplying the weight of ethylene in 1.0 ml of pure ethylene gas by the fraction of ethylene in the root gas sample

$$(1.15 \times 10^{-3} \text{ g } C_2H_4) \times \frac{1.52 \text{ } (\mu l/l)}{10^6 \text{ } (\mu l/l)}$$

$= 1.76 \times 10^{-9}$ g (1.76 nanograms) ethylene in 1.0 ml of root gas sample. This was the weight of ethylene in a 1.0 ml sample of gas from the roots of control plants in experiment 5 run 1-1. The total gas extracted from these roots was 1.03 ml so that multiplying this volume by the weight of ethylene in a 1.0 ml sample gave the total weight of ethylene extracted from these roots, 1.81 nanograms. This value was divided by the total weight of the

roots from which it had been extracted (5.48 g) to yield a concentration of 0.34 nanograms ethylene per gram of root. Gas concentrations were in this way normalized for comparisons across experiments. Ethane concentrations were calculated similarly by substituting in the gram molecular weight for ethane (30.08 g/mole) where appropriate.

5-6. Ethane and Ethylene Concentrations of Susceptible and Resistant Roots

Experiment 1 Ethylene

In experiment 1, ethylene concentrations of control and infested roots were quite variable with time (Fig. 19A). Concentrations as low as 0.023 ng C_2H_4/g root and as high as 0.26 ng C_2H_4/g root were found. These variations in concentration from run to run were roughly parallel in both control and infested plants. This indicates that some common factor affected all plants. Effects of root knot nematode infestation on ethylene concentrations should be evident as differences between control and infested concentrations. Graphical representation of percentage differences revealed that control concentrations were more often greater than infested concentrations, the exception to this being found at days 1½ and 36 (Fig. 19B). Linear regression analysis determined no correlation of increasing or decreasing percentage differences with time and a paired t-test determined the mean difference to be +0.2% which was not significantly different from zero.

Ethane

Concentrations of ethane from both control and infested roots though varying somewhat from run to run showed a general tendency to decrease with time (Fig. 20A). Ethane concentrations from both control and infested plants generally varied in parallel. Concentrations ranged from 0.29 to 4.37 ng

C_2H_6/g root. Comparison of control and infested ethane concentrations using percentage differences is shown in Figure 20B. Initially control levels were greater but as the experiment progressed, infested levels tended to become greater. However, a linear regression analysis determined that this trend was not significant. Paired t-test analysis of the mean percentage difference determined that it was not significantly different from zero.

Experiment 2 Ethylene

Ethylene concentrations for the first 12 days of experiment 2 were consistent around 0.1 ng C_2H_4/g root. These increased abruptly at day 14 and assumed levels in the range of 0.3-0.76 ng C_2H_4/g root for the remainder of the experiment (Fig. 21A). The pattern was similar for both control and infested plants. Graphical representation of the percentage differences between control and infested concentrations is seen in Figure 21B. Patterns indicating a linear trend to increasing positive (control larger) or negative (infested larger) differences with time are not obvious and linear regression analysis revealed no significant correlations here. A mean percentage difference of + 18% was marginally significant (90%).

Ethane

Concentrations of ethane increased at day 14 of experiment 2 as they did for ethylene, but to a lesser degree (Fig. 22A). Again daily variations in ethane levels were largely similar for both control and infested roots. The pattern of these variations over the course of the experiment was similar to that for ethylene (Figs. 21A and 22A). A range in levels from 0.73 to 4.21 ng C_2H_6/g root was encountered. A trend of percentage differences from positive to negative is suggested by Figure 22B. This indicates that control concentrations initially larger than infested concentrations were about

equal by the termination of the experiment. Linear regression analysis determined that this trend was only marginally significant (90%). A paired t-test analysis of the data determined the mean difference of +15% was marginally significantly different from zero. This positive percentage difference indicates that overall ethane concentrations were greater in control than infested roots.

Experiment 3 Ethylene

Control and infested ethylene concentrations exhibited similar patterns of variation over the course of experiment 3 (Fig. 23A). Relatively high concentrations were found in all roots analyzed in the first two days however by day 4 ethylene concentrations had assumed lower levels which were relatively consistent for the remainder of the experiment. A range in concentrations of 0.023 to 0.63 ng C_2H_4/g root was found. Percentage differences tended to increase in a positive direction over the duration of the experiment indicating that control concentrations were increasing relative to infested concentrations (Fig. 23B). A marginally significant correlation (90%) of this tendency to increase with time was determined by linear regression analysis. Paired t-test analysis determined that the mean percentage difference of +47% was significantly different from zero. Thus, roots of control plants had significantly greater ethylene concentrations than roots of infested plants and this difference was increasing with time.

Ethane

Considerable daily variations in ethane concentrations were found over the course of experiment 3 (Fig. 24A). A range of 0.25 to 2.28 ng C_2H_6/g

root was found. In this experiment, ethane and ethylene levels appear to have varied together (Figs. 23A and 24A). Percentage difference calculations revealed that control ethane concentrations were consistently greater than infested (Fig. 24B). A tendency for these differences to increase with time is seen with the exception of an atypically large day 9 percentage difference (+510%). Consequently linear regression analysis determined that this trend was not significant. The mean control ethane concentration was 91% greater than the mean infested ethane concentration which was significant.

Experiment 4 Ethylene

The pattern of ethylene concentrations versus time of experiment 4 resistant plants was markedly similar to that of experiment 3 susceptible plants (Fig. 25A). Relatively high ethylene concentrations were found for the first 2 days in both control and nematode-treated plants with no consistent differences between them. By day 4, ethylene concentrations had decreased considerably and were consistently in the range 0.048 to 0.15 ng C_2H_4/g root for the remainder of the experiment. A range of 0.30 to 0.40 ng C_2H_4/g root was found for days 0 to 2. Similar concentrations were found in the roots of the susceptible plants of experiment 3.

Graphical representation of the percentage differences of ethylene in control and nematode-treated plants showed a random orientation about zero (Fig. 25B). There was no linear correlation of these differences with time and paired t-test analysis determined the mean percentage difference (-5%) was not significantly different from zero. Thus there were no significant differences between ethylene concentrations of control and nematode-treated plants in experiment 4.

Ethane

Ethane concentrations from control and nematode-treated plants were similar and in the range of 0.28 to 1.22 ng C_2H_6/g root (Fig. 26A). This range was comparable to that obtained from the susceptible plants of experiment 3. Figure 26B reveals that no linear changes of percentage differences occurred with time. Linear regression analysis substantiated this observation. The mean ethane concentration of control plants was determined to be 5% greater than that of infested plants. This was not a significant difference.

Experiment 5 Ethylene

Ethylene concentrations in experiment 5 ranged from 0.21 to 0.93 ng C_2H_4/g root with most concentrations in the range of 0.3 to 0.6 ng C_2H_4/g root (Fig. 27A). Consistent differences of ethane concentration between control and infested susceptible plants were not apparent. Graphing the percentage differences (Fig. 27B) further illustrated the lack of any consistent pattern here and linear regression analysis determined no significant correlation existed between ethylene concentration and time. The mean percentage difference was -17% but this was not significant as determined by the paired t-test.

Ethane

Ethane concentrations from control and infested plants in experiment 5 are plotted against time in Figure 28A. A dramatic increase in ethane concentrations from control and infested plants is seen over the 30 day experimental time course. Concentrations ranged from 0.47 to 5.43 ng C_2H_6/g root. No significant correlation of changing ethane percentage differences

with time existed as determined by linear regression analysis of the data in Figure 28B. Paired t-test analysis determined that the mean ethane percentage difference of -10% was not significantly different from zero.

Experiment 6 Ethylene

A range of 0.19 to 0.60 ng C_2H_4/g root was found for the resistant plants of experiment 6 (Fig. 29A). As this range indicates concentrations were fairly consistent and daily variations minimal. The variation of concentrations with time was similar to that obtained with susceptible plants in experiment 5. Experiments 5 and 6 were run concurrently. Analysis of the percentage differences revealed that no linear changes with time occurred (Fig. 29B). The mean ethylene concentration was 3% larger in control roots as compared to nematode treated roots. This value was not significantly different from zero.

Ethane

Root concentrations of ethane increased considerably over the course of experiment 6 (Fig. 30A). Levels of 0.37 to 4.60 ng C_2H_6/g root were obtained. The pattern and range of concentrations found were similar to those of experiment 5. Ethane concentrations of control and nematode treated plants were similar up to day 13. After day 13, consistently greater ethane concentrations were obtained from control plants (Fig. 30B). Linear regression analysis determined no significant correlation between changing percentage differences and time. The mean ethane concentration was 29% greater in control plants and this value was significantly different from zero as determined by the paired t-test.

Figures 19-30A and B.

Changes in ethylene and ethane root concentrations with time. Each point on the graphs is the mean value obtained from two experimental determinations. Graphs 19-30A show the root concentrations of ethylene or ethane in nanograms per gram root versus time post-infestation for experiments 1-6. (● control, Δ infested) Graphs 19-30B show the percentage differences of control versus infested root gas concentrations plotted against time post-infestation for experiments 1-6. Percentage differences were calculated with the large concentration for each run, control or infested, as the numerator. 100% was then subtracted to give the difference. Control concentrations larger had percentage differences designated positive and infested concentrations larger had percentage differences designated negative. Since each point was the mean value of two determinations, the range in percentage difference is indicated. Absence of range bars indicates that the value was obtained from a single experimental determination. A single range bar through the centre of a point indicates identical values were obtained from both determinations. The mean percentage difference is indicated by a horizontal dashed line.

FIGURE 19. EXPERIMENT I ETHYLENE

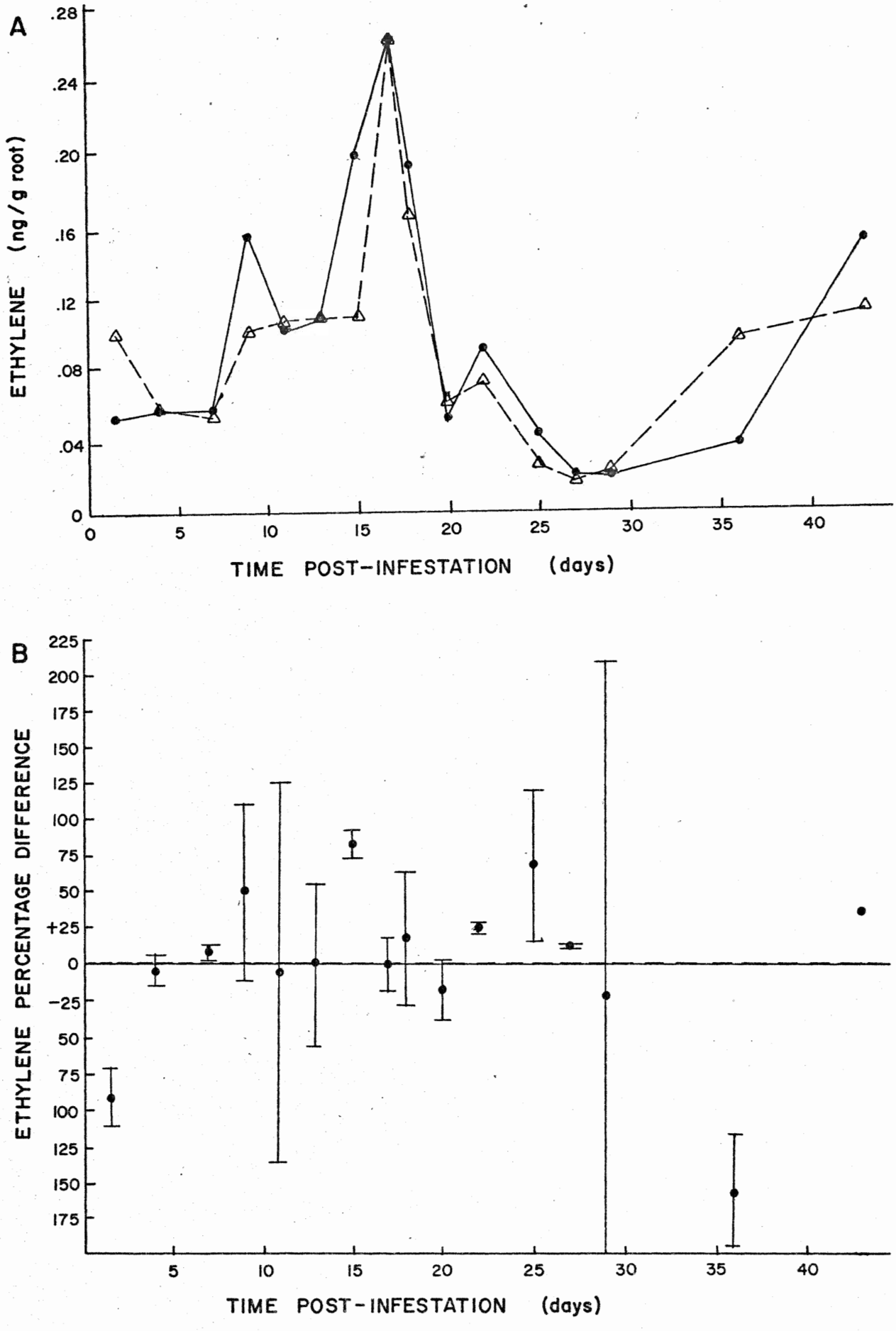


FIGURE 20. EXPERIMENT I ETHANE

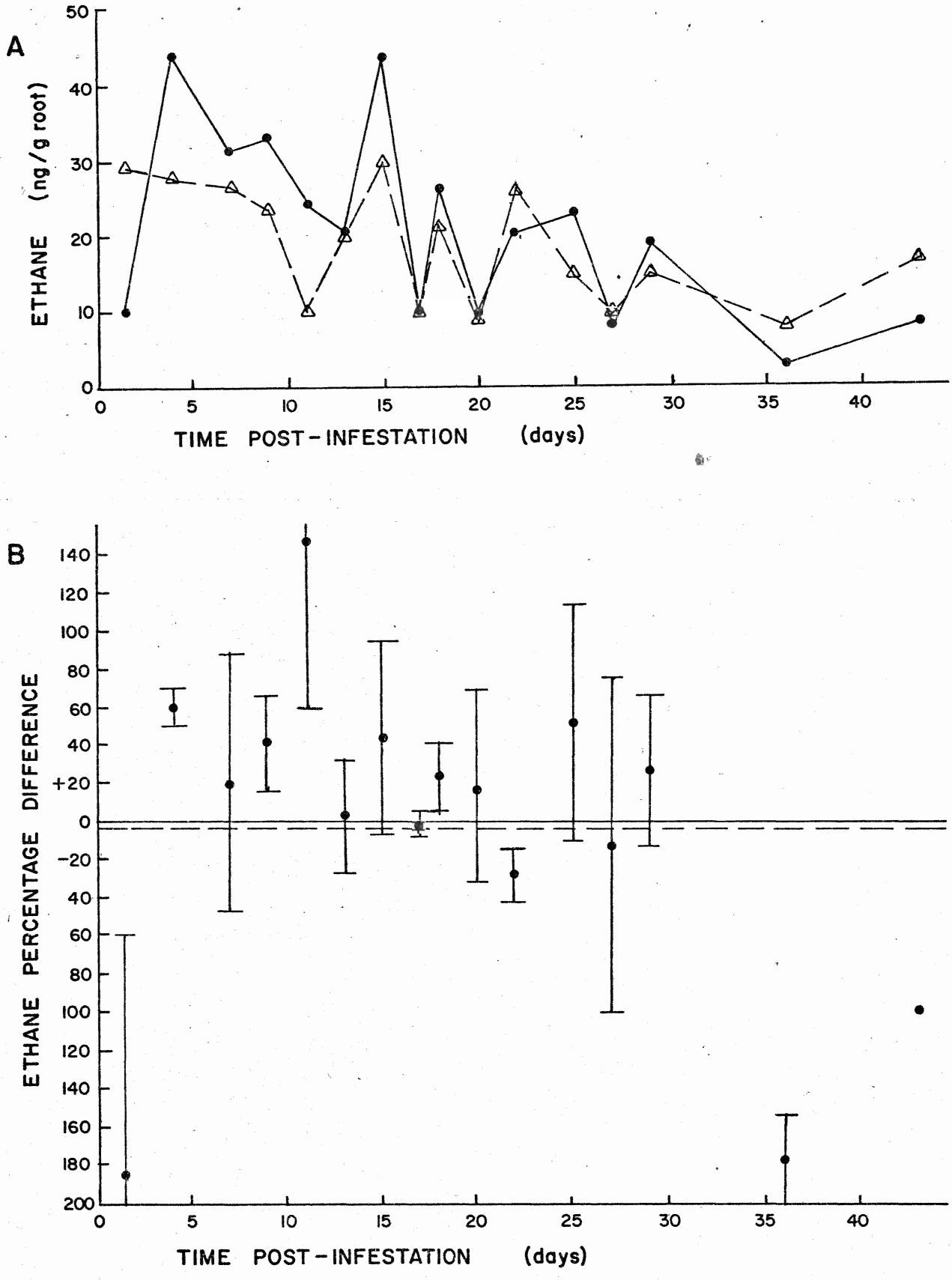


FIGURE 21. EXPERIMENT 2 ETHYLENE

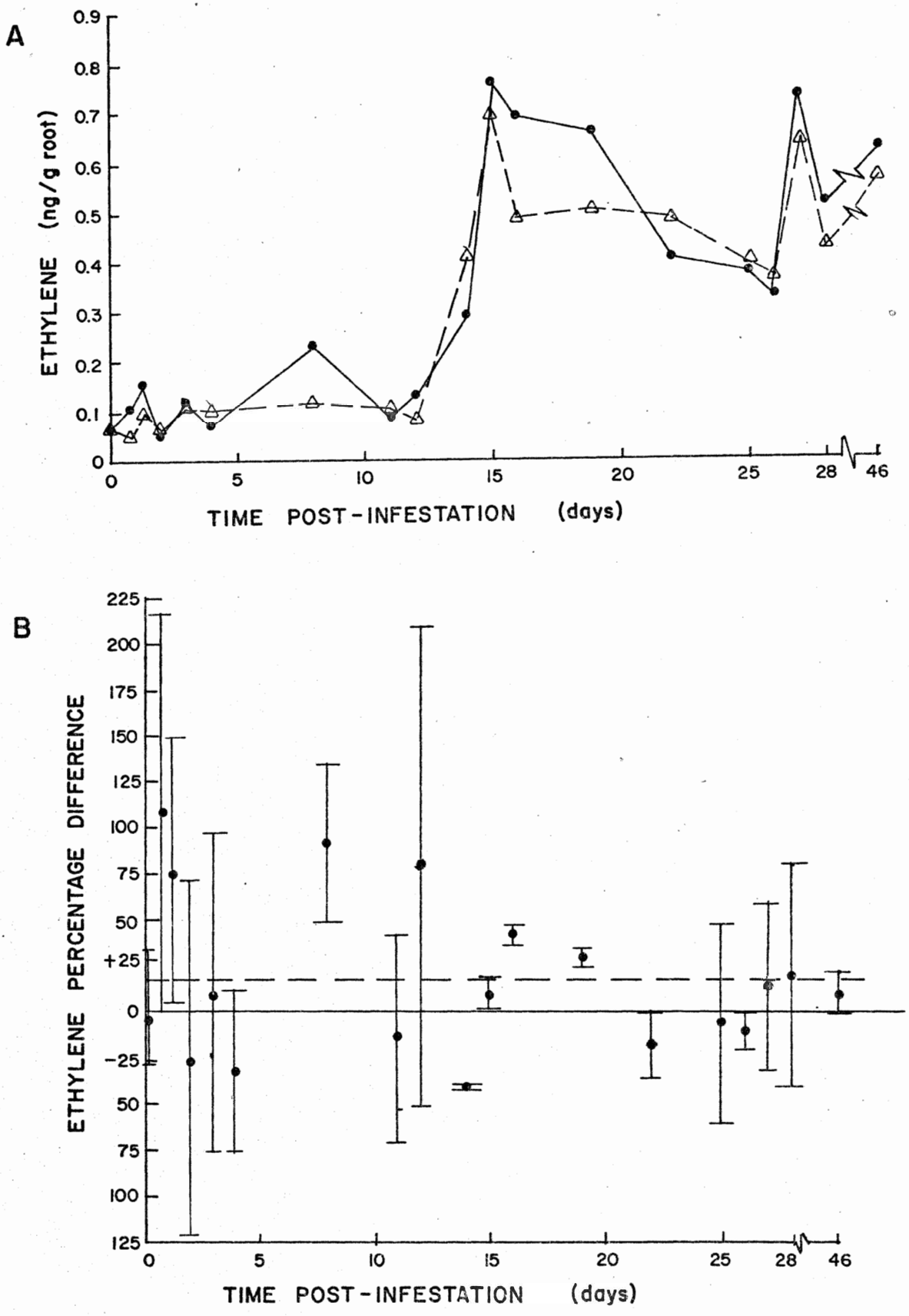
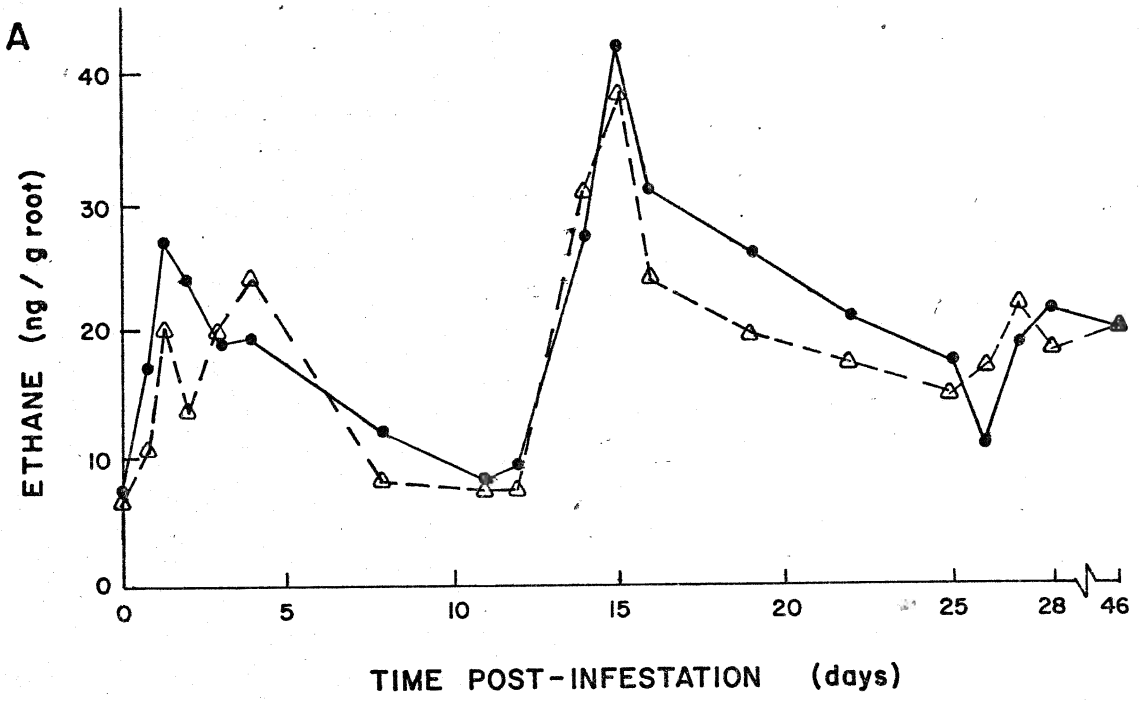


FIGURE 22. EXPERIMENT 2 ETHANE



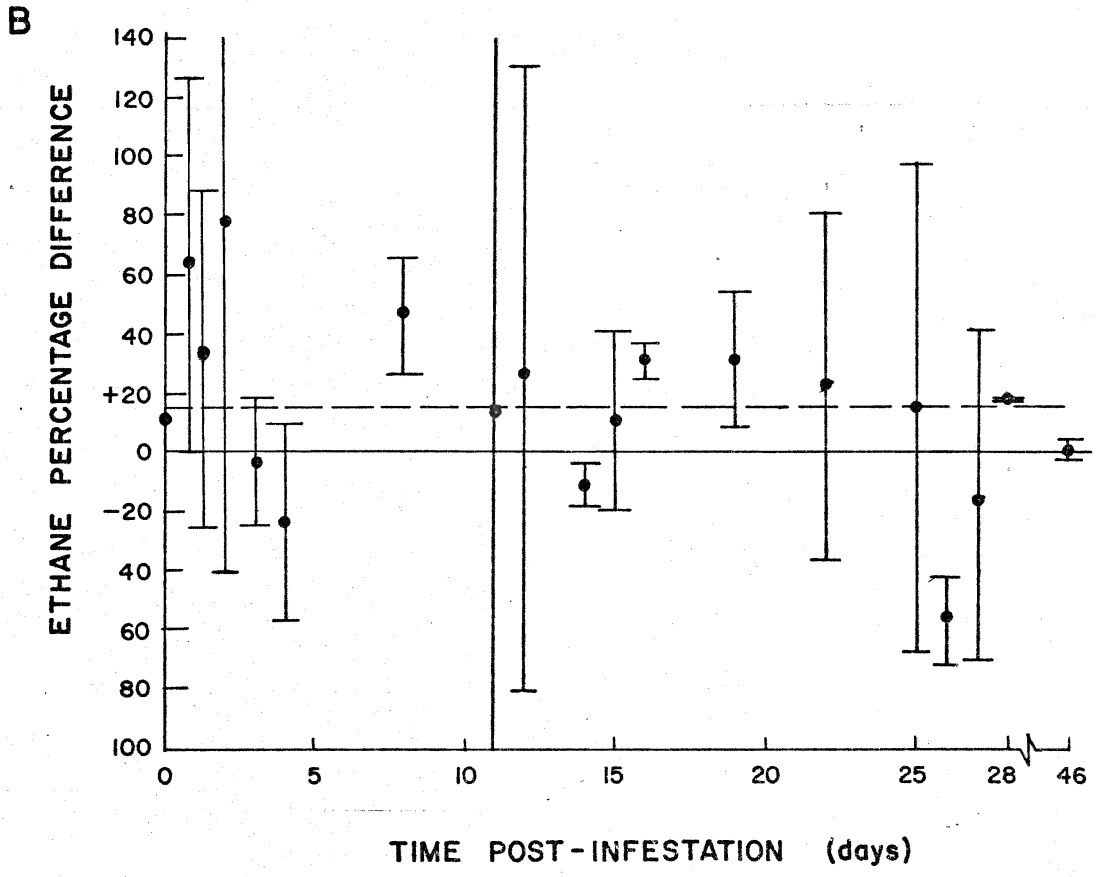


FIGURE 23. EXPERIMENT 3 ETHYLENE

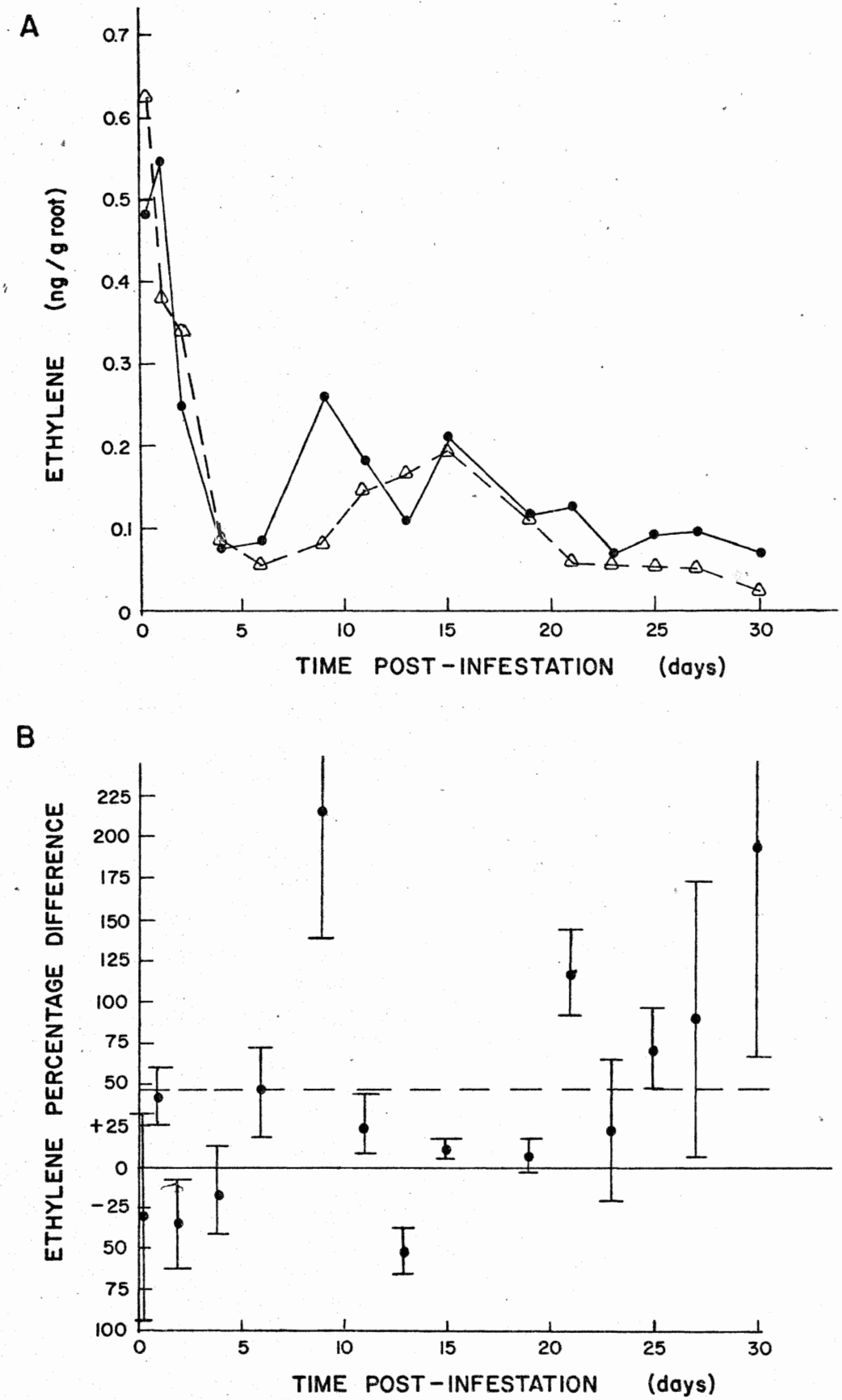


FIGURE 24. EXPERIMENT 3 ETHANE

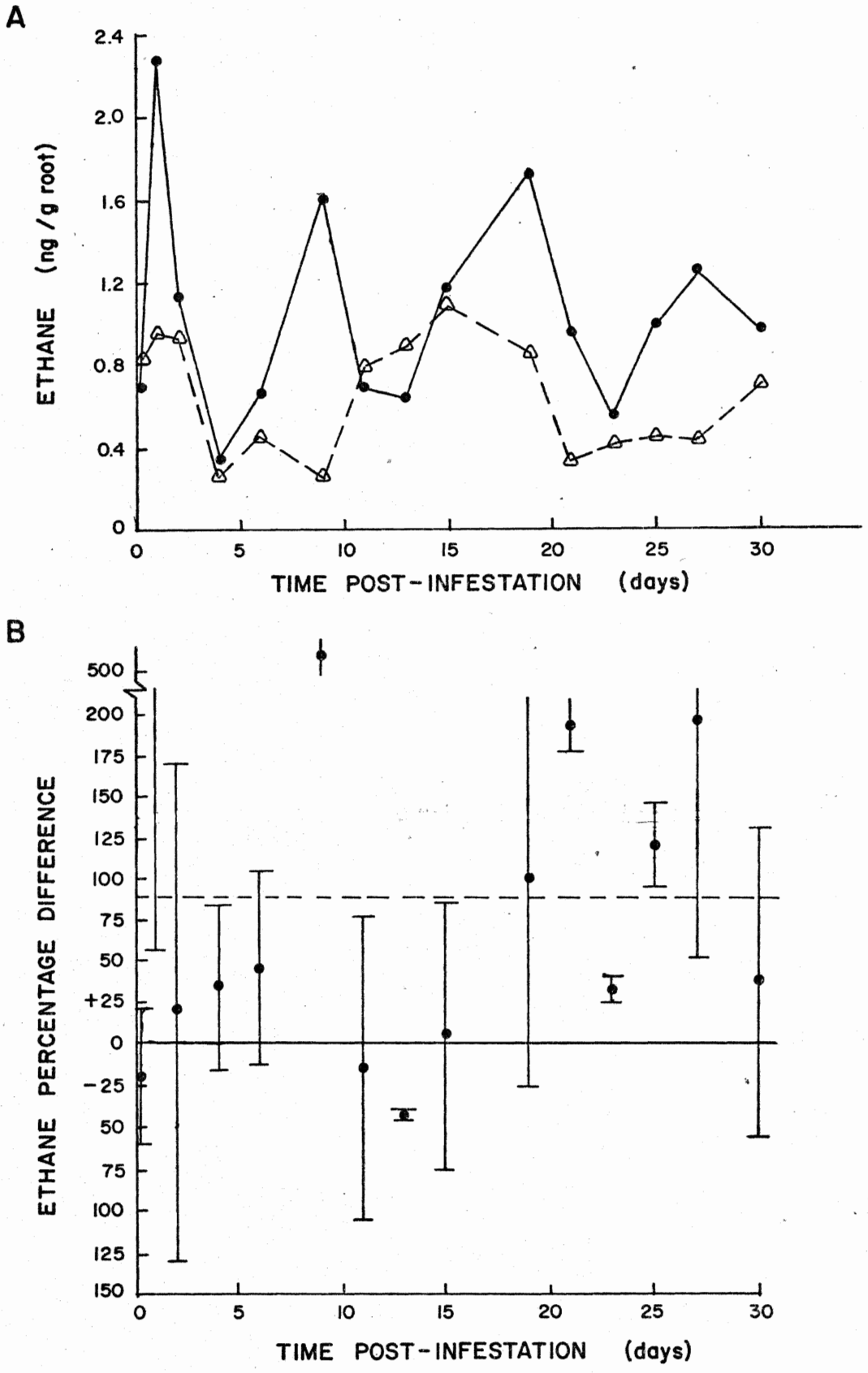


FIGURE 25. EXPERIMENT 4 ETHYLENE

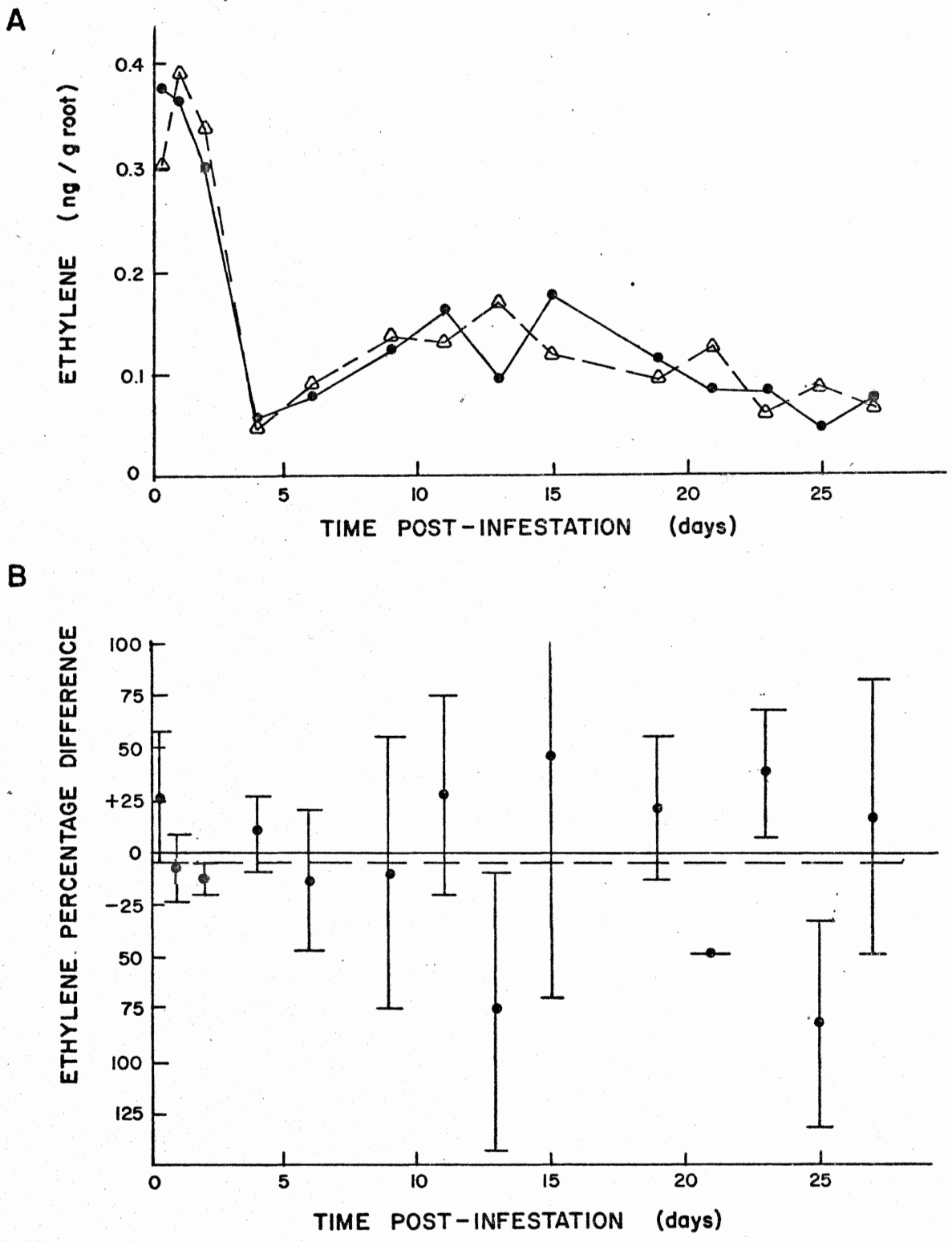


FIGURE 26. EXPERIMENT 4 ETHANE

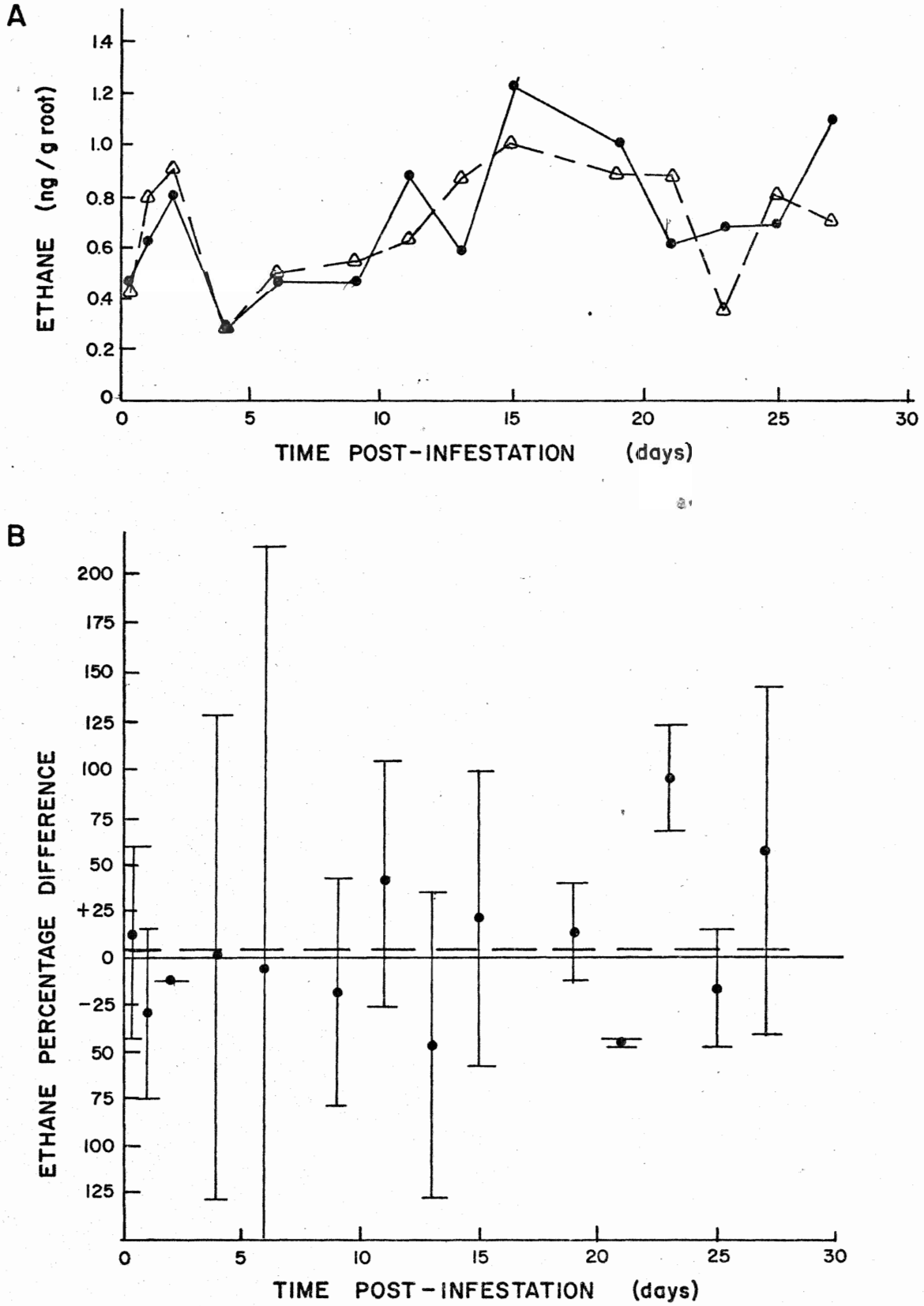


FIGURE 27. EXPERIMENT 5 ETHYLENE

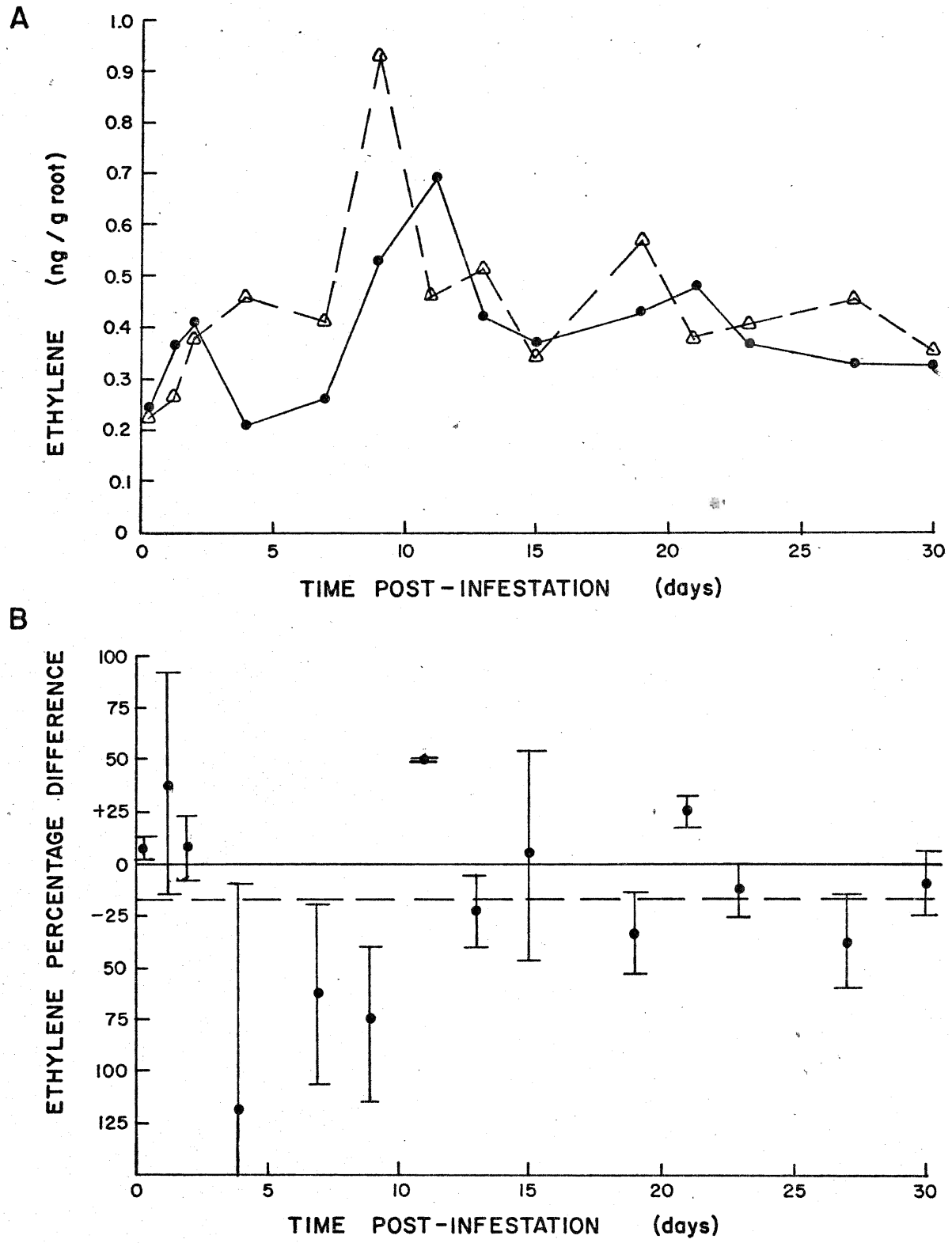


FIGURE 28. EXPERIMENT 5 ETHANE

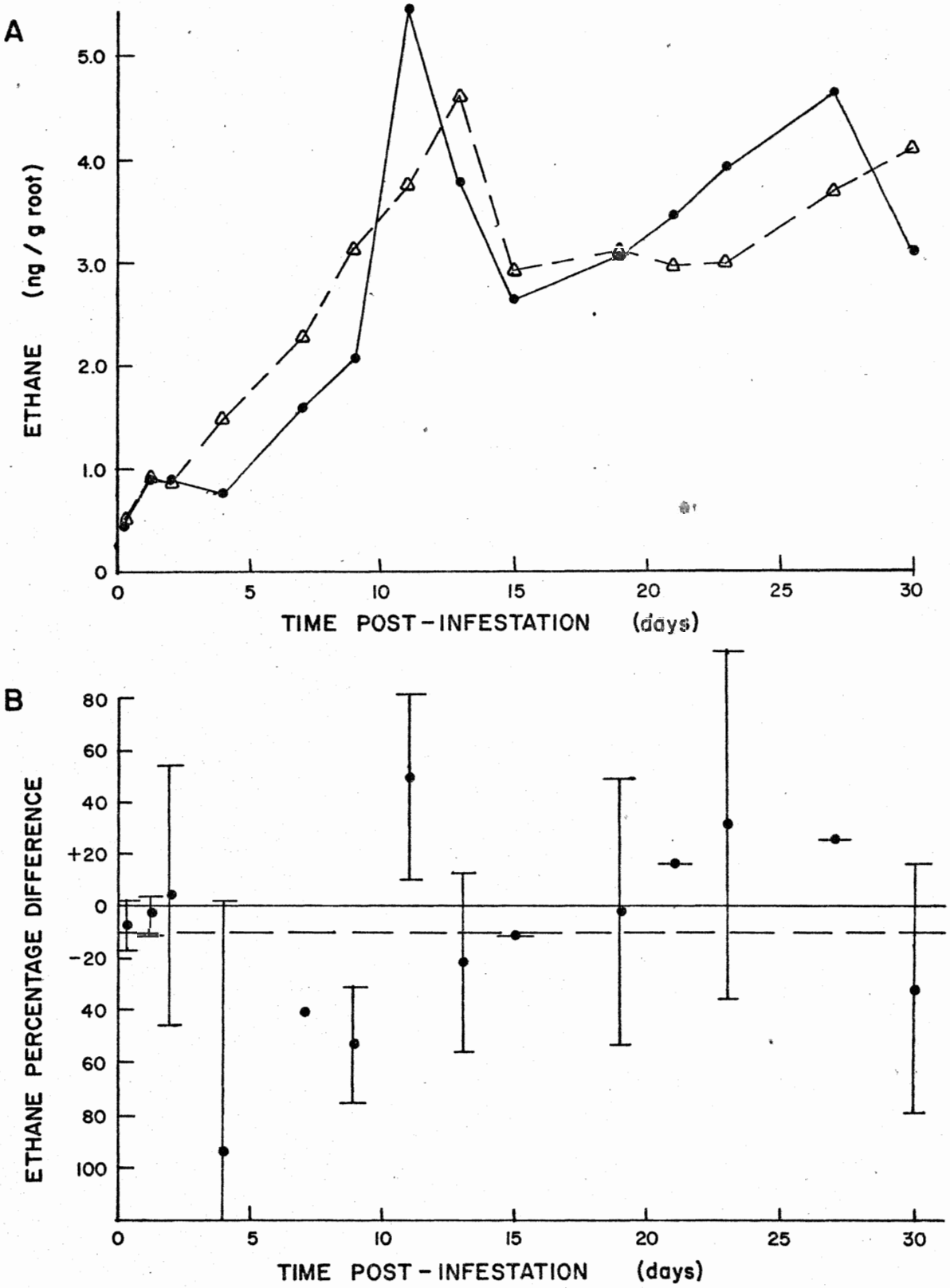
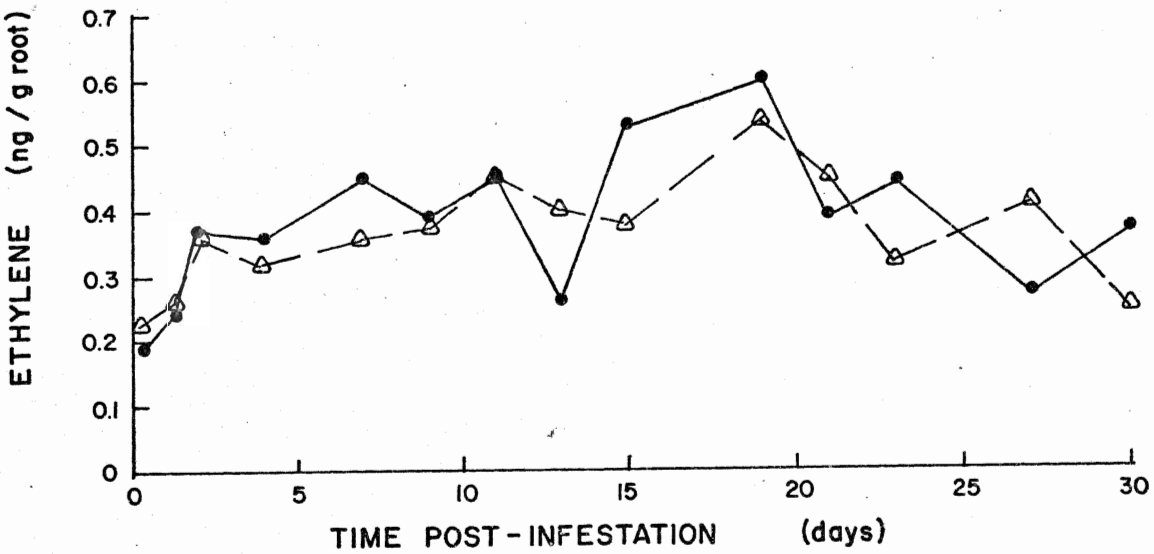


FIGURE 29. EXPERIMENT 6 ETHYLENE



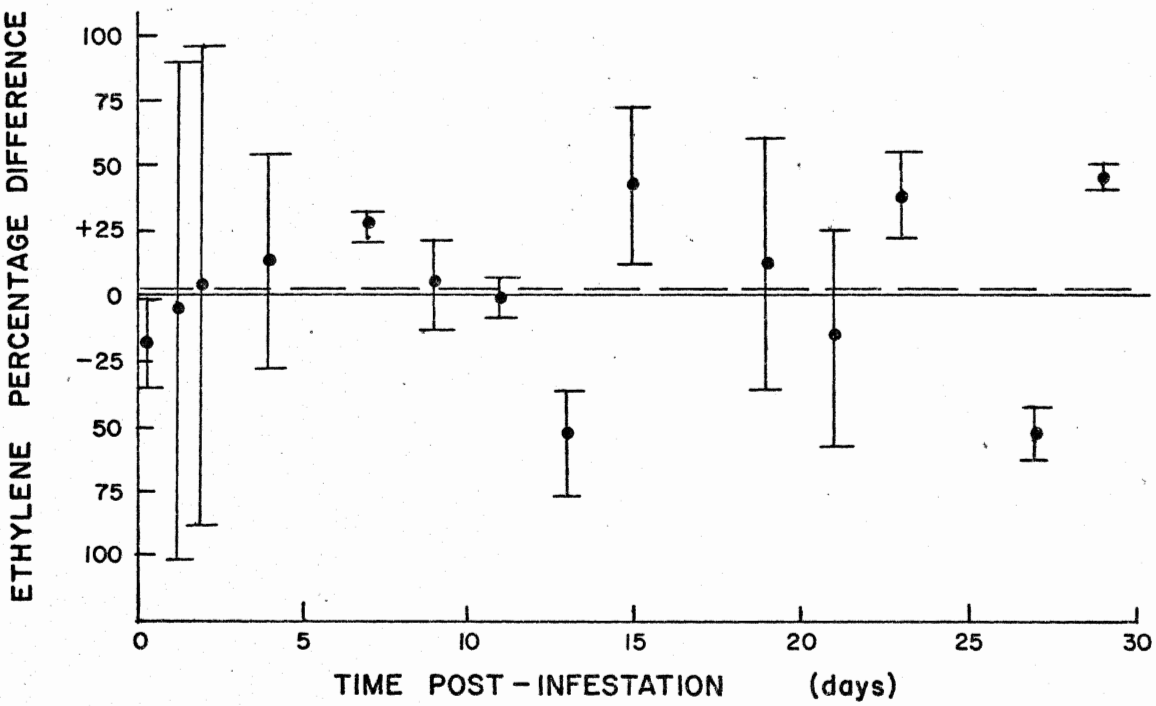
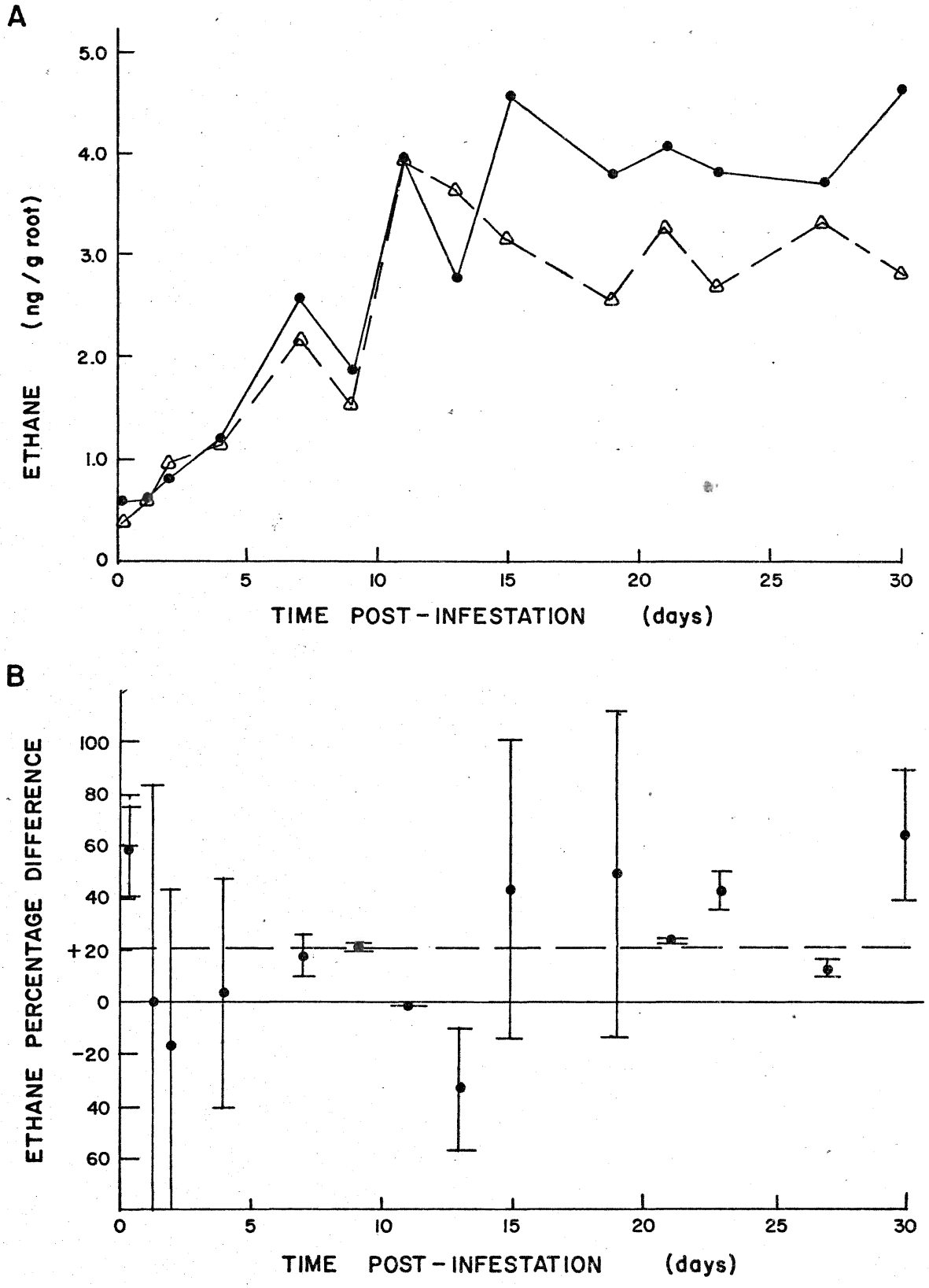


FIGURE 30. EXPERIMENT 6 ETHANE



5-7. Ethane/Ethylene Root Contration Ratios

Ethane and ethylene concentrations for the six experiments indicated considerable similarities. Concentrations of ethane were invariably greater than ethylene obtained from the same root gas sample. Concentrations of ethylene and ethane generally varied in parallel with time. To further examine the relationship of these gases in the experimental roots, the ratio of ethane/ethylene was computed for each experimental run. If the relative ethane and ethylene concentrations fluctuated together their ratios would be a constant value around which some random variation would occur. Conversely, if the two gasses varied independently, graphing the gas ratios would indicate variations in ratios with time. The analysis also resulted in a comparison of ethane/ethylene ratios for both control and nematode-infested roots.

Results of the calculations are seen in Figures 31-36. In all cases, control and infested ethane/ethylene ratios within an experiment tended to vary in parallel. This enabled general patterns to be assessed for each experiment. Experiment 1 ratios varied widely over a range of 4 to 104. Ethane/ethylene ratios for experiment 2 displayed a statistically significant trend to decrease over the experimental time period. In contrast, ratios consistently increased over the time course of experiments 3-6. Linear regression analysis of both control and infested ratios determined that the trend to increase with time in experiments 3 to 6 was significant.

To determine any effects of root knot nematode infestation differences between control and infested ratios were calculated. Linear regression and paired t-test analyses were performed on these differences. Linear regression analysis determined that a significant change in differences with time occurred in experiments 4 and 6, both of which involved resistant

Figures 31-36. Ethane/ethylene ratios versus time post-infestation for experiments 1-6.

Ratios were calculated by dividing the mean ethane concentration by the mean ethylene concentration for each run of an experiment for both control and infested plants. (● control, Δ infested).

FIGURE 31. EXPERIMENT 1 ETHANE/ETHYLENE RATIOS

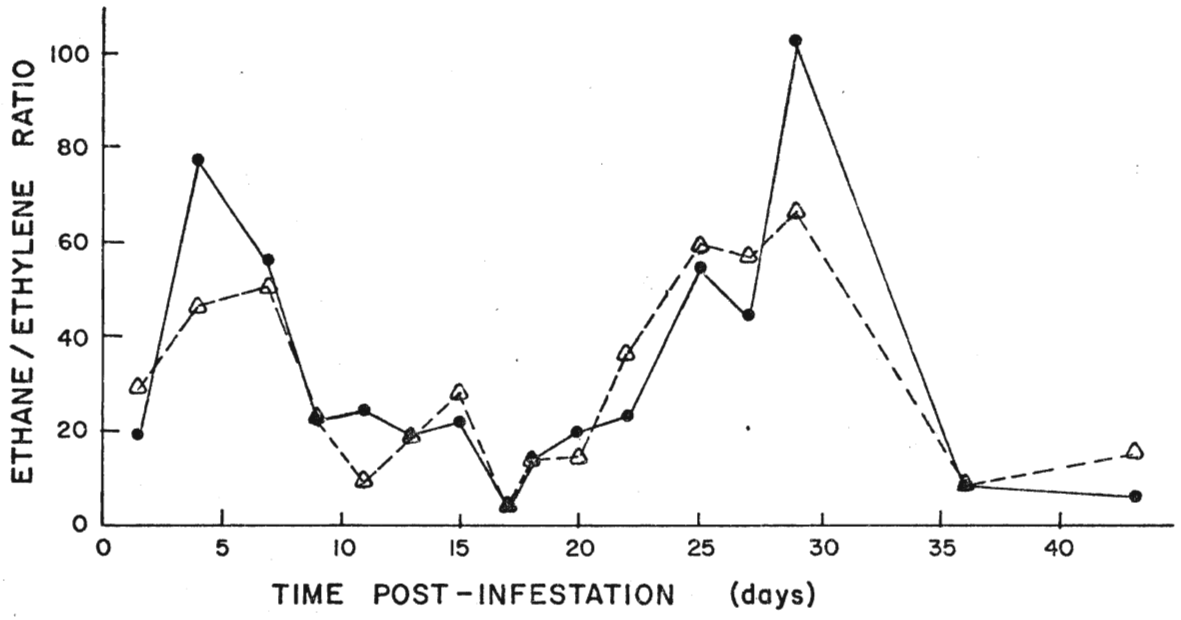


FIGURE 32. EXPERIMENT 2 ETHANE/ETHYLENE RATIOS

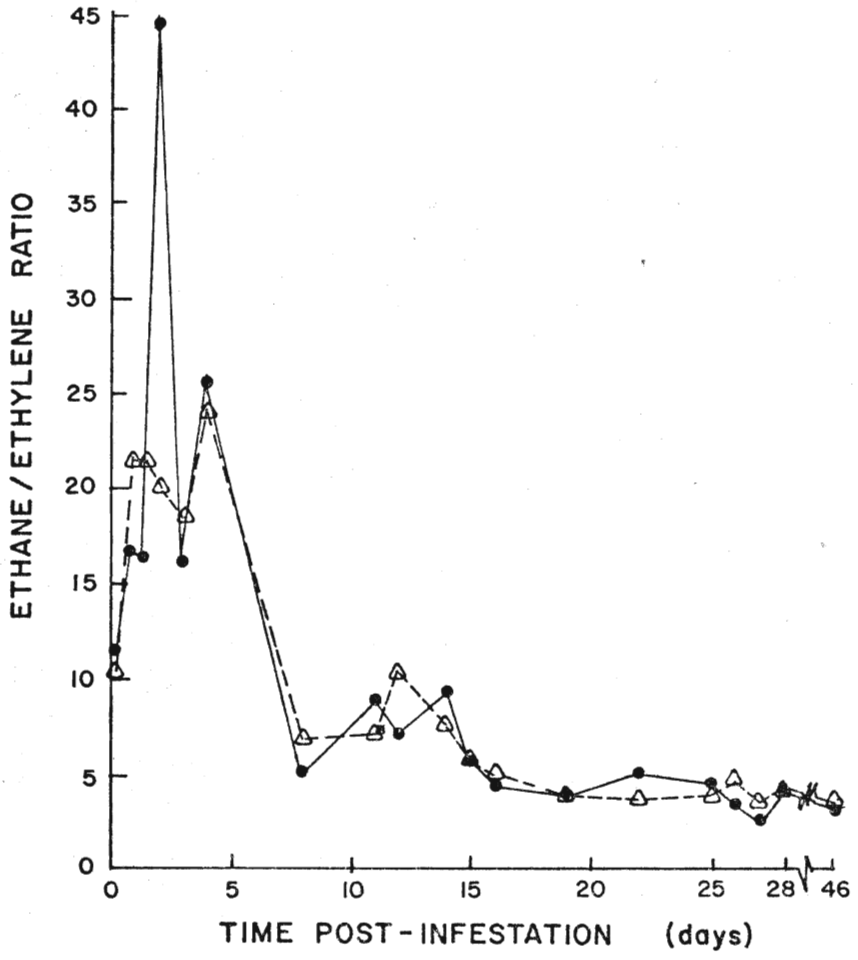


FIGURE 33. EXPERIMENT 3 ETHANE/ETHYLENE RATIOS

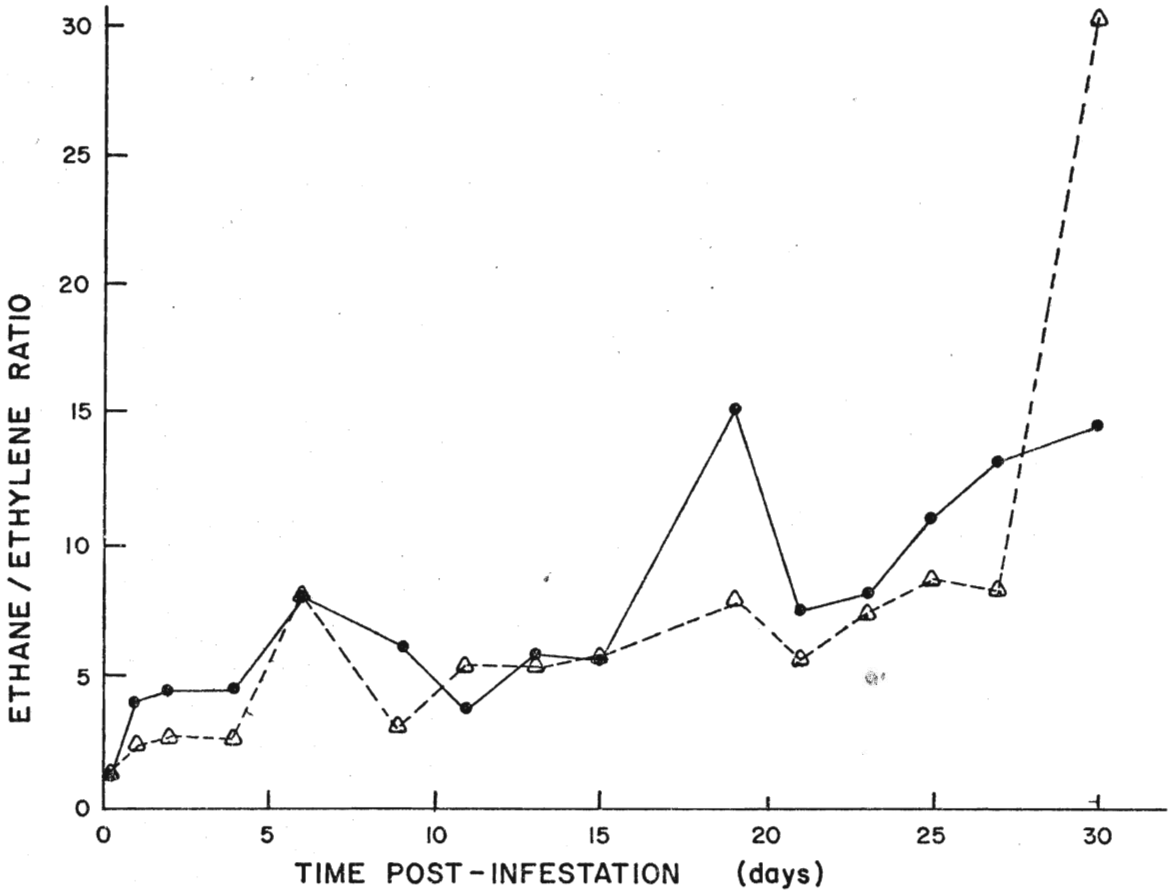


FIGURE 34. EXPERIMENT 4 ETHANE/ETHYLENE RATIOS

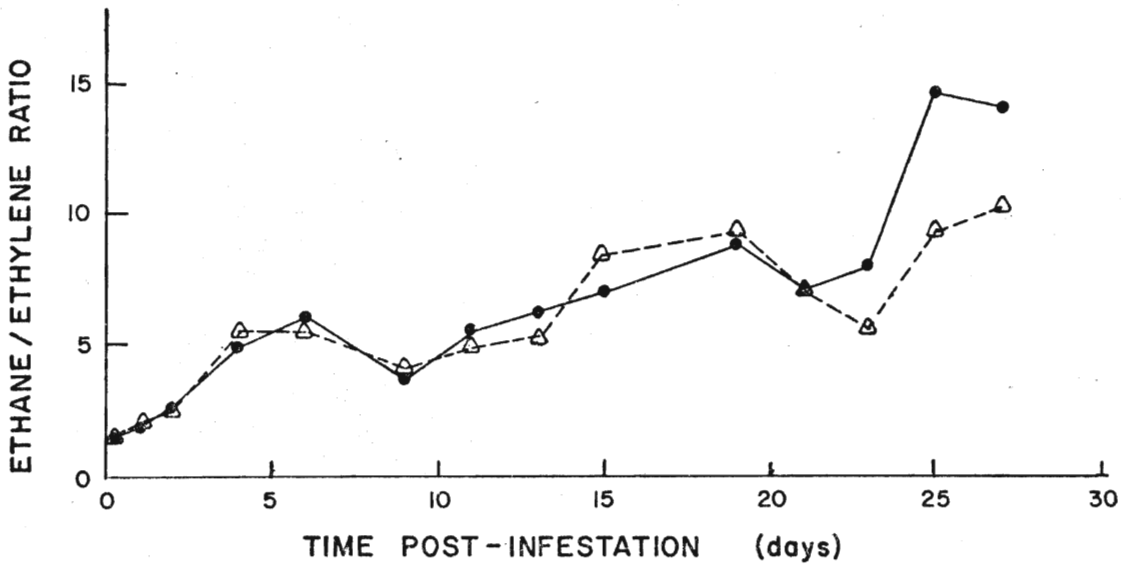


FIGURE 35. EXPERIMENT 5 ETHANE/ETHYLENE RATIOS

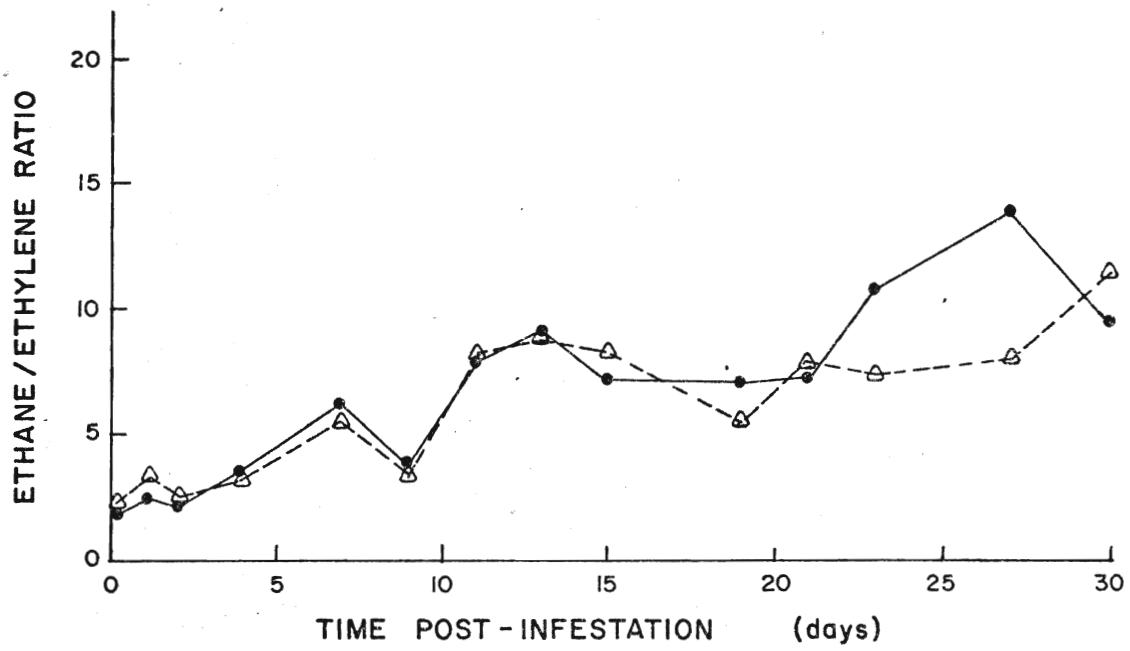
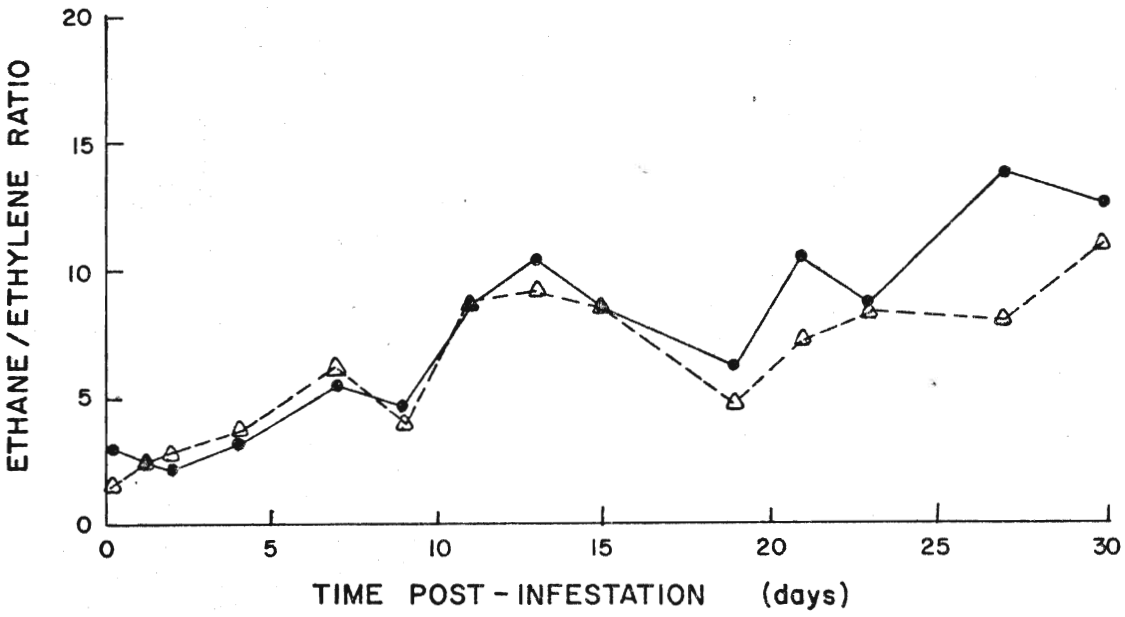


FIGURE 36. EXPERIMENT 6 ETHANE/ETHYLENE RATIOS



plants. In these experiments, the ratios of control roots were increasing as compared to the ratios of nematode-treated roots. No significant correlations were found in any of the experiments involving susceptible plants.

Paired t-test analysis determined that the only experiment in which the mean ethane/ethylene ratio from control plants was significantly different from that of infested plants was experiment 6. In this case, mean control ratios were greater than infested. The resistant plants of experiment 4 were unique in that there was no significant difference between their mean control and infested ratios, however a significant correlation of changing differences with time was found.

5-8. Correlation of Gas Concentration with Root Weights

Root weights of infested plants tended to increase more rapidly than those of control plants due to the formation of galls. In a number of experiments, gas concentration when expressed on a per gram root weight basis decreased with time. These two trends suggest that the total amount of root ethylene and ethane did not increase in proportion to increases in root weight. This possibility was investigated by linear regression analysis on ethane and ethylene concentrations expressed in nanograns per gram root versus their corresponding root weights. If these two parameters increased in direct proportion to each other, no significant correlation of increasing or decreasing gas concentrations versus root weights would be found. Graphically this situation would yield a horizontal line parallel to the x-axis (root weight). Linear regression analysis determined significant linear deviations from the line of zero slope.

Table 4. Summary of Ethane/Ethylene Root Concentration Ratios.

Experiment	Pattern of ratios over time for both control and infested plants[1]	Differences control minus infested ratios	
		Linear Regression[2]	Paired t-test[3]
1. Susceptible plants	-varied widely -no consistent trends with time	NS	NS
2. Susceptible plants	-decreased with time -significant for both control and infested	NS	NS
3. Susceptible plants	-increased with time -significant for both control and infested	NS	NS
5. Susceptible plants	-increased with time -significant for both control and infested	NS	NS
4. Resistant plants	-increased with time -significant for both control and infested	-significantly increasing with time	NS
6. Resistant plants	-increased with time -significant for both control and infested	-significantly increasing with time	significantly different C > I

1. Significance of patterns of ratios with time were determined by linear regression analysis of control and infested data individually.

2. Linear regression analysis of differences (control minus infested) with time determined if a significant linear increase or decrease in magnitude of these differences occurred with time.

3. Paired t-test analysis determined whether the mean ratios of control and infested roots were significantly different.

NS indicates not significant.

Significant correlations between ethylene concentration and root weights were obtained for infested plants of experiments 1 and 3, and control plants of experiment 6 (Table 5). The line of best fit in each of these cases had a negative slope which indicated that as root weights increased, the increase in total ethylene in these roots was less than the proportional increase in root weight. Where no linear correlation was found it was assumed that there was no significant difference between the proportional increase in total root ethylene and root weight. In half the experiments with susceptible plants a negative correlation was found for infested roots while their controls showed no linear correlation between root concentration of ethylene and root weights.

Experiments showing significant linear correlations outnumbered experiments showing no correlation by two-to-one for ethane concentrations plotted against their corresponding root weights. Significant correlations were obtained for experiments 1, 3 and 6 control and infested data, experiment 5 infested and experiment 4 control. In all cases the slope of the line of best fit was negative.

The results indicate that total root gas volumes increased either proportionally with increasing root weight (no significant correlation) or increased less than the relative increase in root weight (significant negative correlation). This latter trend was obtained more often for ethane than for ethylene and in some experiments where ethylene had no significant correlation, ethane data from the same roots exhibited a negative correlation (experiments 3, 4 control; experiments 5, 6 infested). Significant negative correlations were obtained for control and infested ethane data in equal frequencies.

Table 5. Linear Regressions of Gas Concentrations Versus Root Weights

Gas	Experiment	Phenotype of Plants	Significance of Fit of Linear Regression Control	Slope	Infested	Slope
Ethylene	1	Susceptible	NS		90%	-0.0086
	2		NS		NS	
	3		NS		99%	-0.019
	5		NS		NS	
	4	Resistant	NS		NS	
	6		95%	-0.029	NS	
Ethane	1	Susceptible	99%	-0.22	99%	-0.16
	2		NS		NS	
	3		90%	-0.18	90%	-0.023
	5		NS		90%	
	4	Resistant	99%	-0.060	NS	
	6		99%	-0.37	95%	-0.14

NS-indicates no significant correlation

5-9. Magnitude of Nematode Infestations

The extent and severity of nematode infestation was determined by the size of the root system at infestation and the amount of infectious second stage larvae applied to it. Plants in experiment 1 were grown in 5 cm square pots whereas all other experimental plants were grown in 10 cm square pots. Infectious second stage larvae of M. incognita were applied in doses per plant of 2,000 experiment 1, 10,000 experiment 2 and 17,000 to 18,000 experiments 3 to 6. The extent of nematode infestation was quantified by estimating the percentage of total mass of a root system that was gall (Figure 38, plates 1-6).

The extent of infestation of experimental root systems is shown in Figure 37. First visible signs of galls occurred at day 11 (post-infestation in experiment 1 as compared to day 4 in experiments 2 and 5 and day 6 in experiment 3. Galls developed slowly over the time course of experiment 1 and by the last run of this experiment (day 43) galls accounted for an estimated 25% of the total root mass of the infested plants. Galls developed on the roots of experiment 2 plants at a quicker rate than in experiment 1. Levels of 50 to 60% were found over the last three runs of the experiment (days 25, 27 and 46). Development of galls on the roots of experiment 5 plants was initially more rapid and extensive than in experiment 2 but slowed to approximately the same rate of development from day 15 on. At the termination of experiment 5 (day 30) 60% of the total root mass of the plants in that run was estimated to be gall. Galls developed most rapidly on the roots of plants in experiment 3. The most severe infestations were encountered in this experiment with galls accounting for an estimated 80% of the root mass of the plants by the last experimental run (Fig. 38 plate 5).

Figure 37. Rate of gall formation with time.

Roots were examined under a stereomicroscope and the mass of root galled estimated as a percentage of total root mass. Each point is the mean value of all infested roots used in that run.

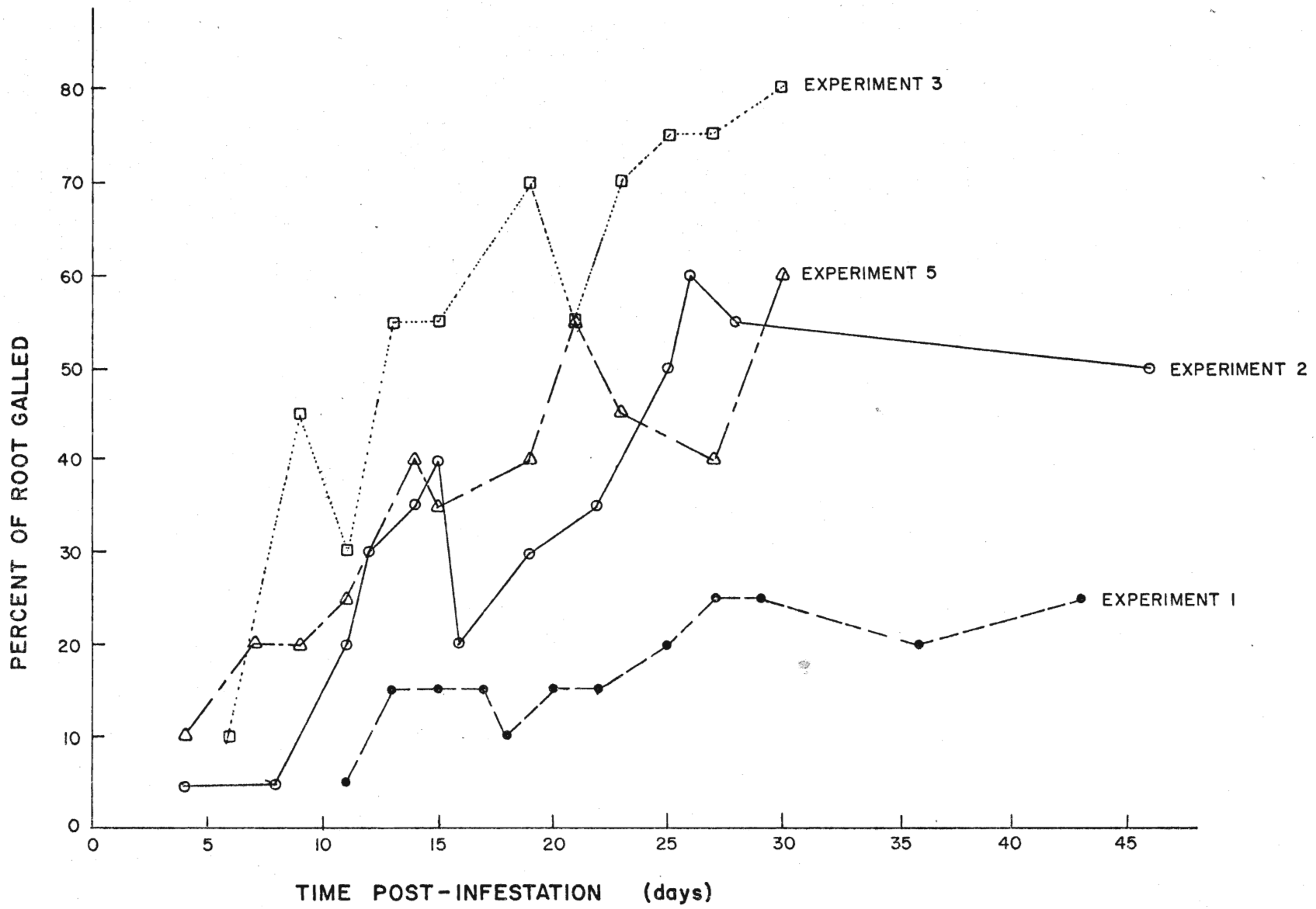

EXPERIMENT 3
EXPERIMENT 5
EXPERIMENT 2
EXPERIMENT 1
PERCENT OF ROOT GALLED
TIME POST-INFESTATION (days)
0
10
20
30
40
50
60
70
80
0
5
10
15
20
25
30
35
40
45

Figure 38. Plates 1-6. Photographs of roots demonstrating severity of *M. incognita* infestation.

A Canon AE-1 35 mm camera and macrolens with Panatomic X asa 64 film was used.

Plate 1. Nematode treated roots of a resistant plant taken from run 9 (day 15) of experiment 6. Roots of plants in this experiment were well developed and extensive. Small dark spots seen on these roots arose from kinks in the rootlets which increased the density of stain and are not small galls. Galls were never encountered in roots of resistant plants.

Plate 2 Root system of a susceptible plant taken from run 8 (day 11) of experiment 2. Galls were visible as small swellings (arrows) and are often continuous for some distance along the rootlets. An estimated 20% of this root mass was gall.

PLATE 1

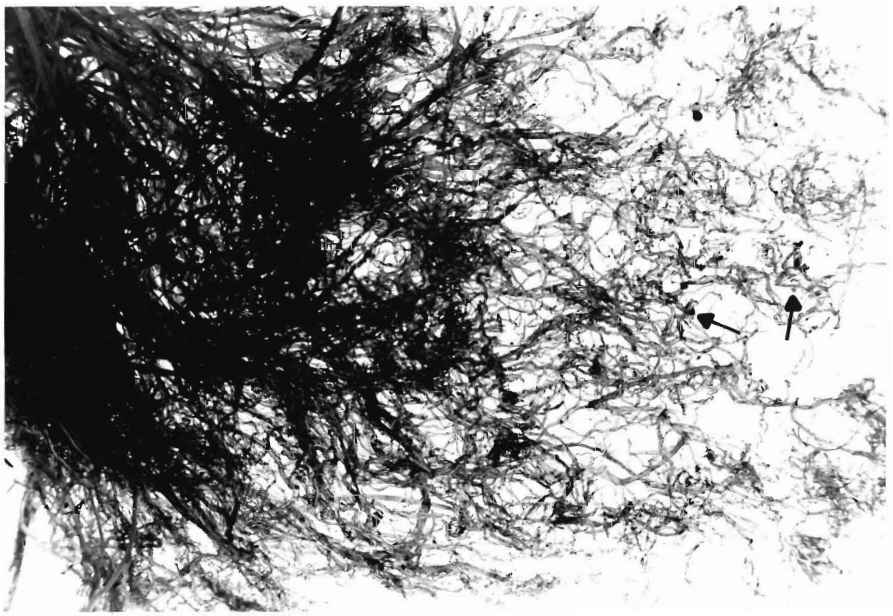


PLATE 2

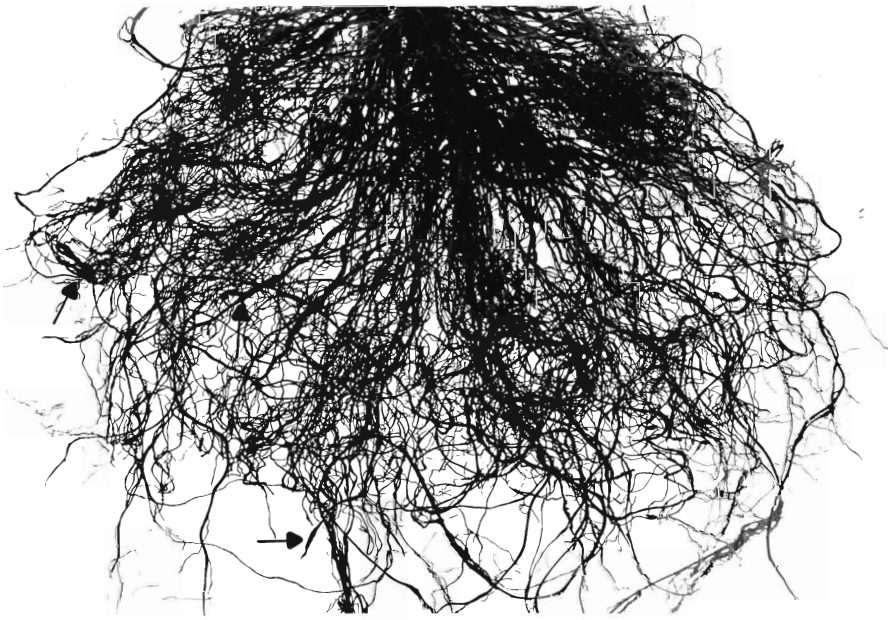


Plate 3 Root system of a plant taken from run 9 (day 15) of experiment 5. Galls are fairly well established (arrows) however much of the root system remains uninfested. An estimated 35% of this root system was gall.

Plate 4 Heavily infested root system taken from run 16 (day 26) of experiment 2. Galls are extensive and well developed accounting for an estimated 55% of this root mass.

PLATE 3

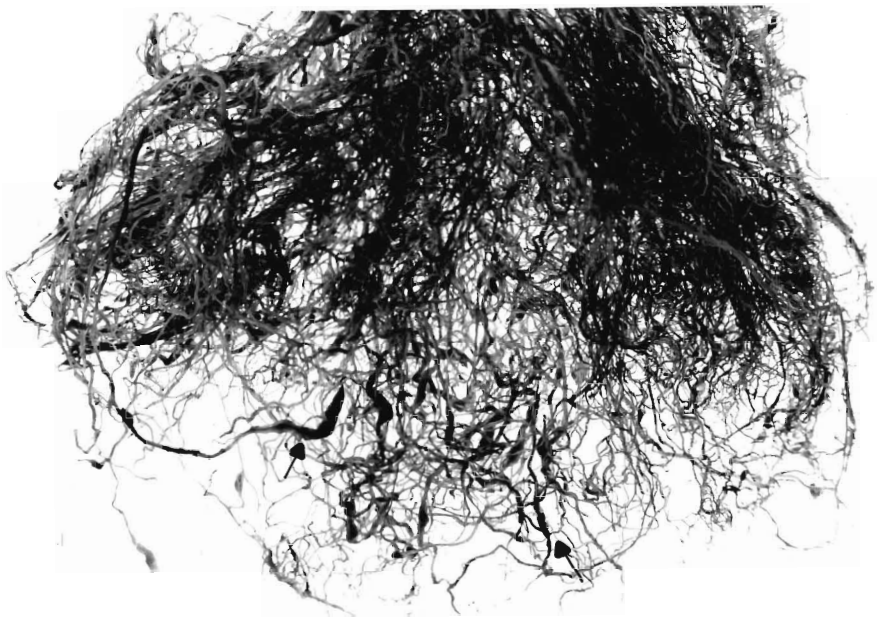


PLATE 4

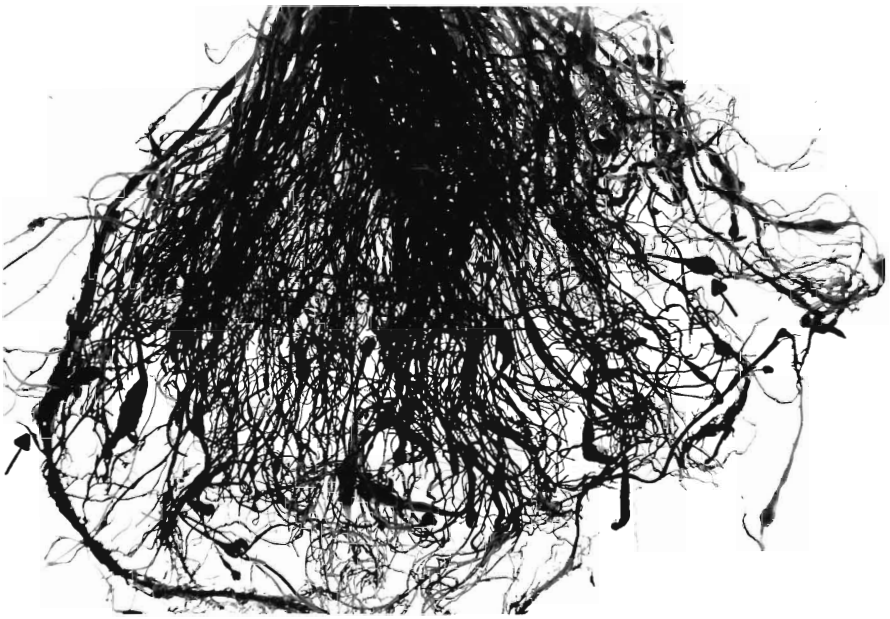
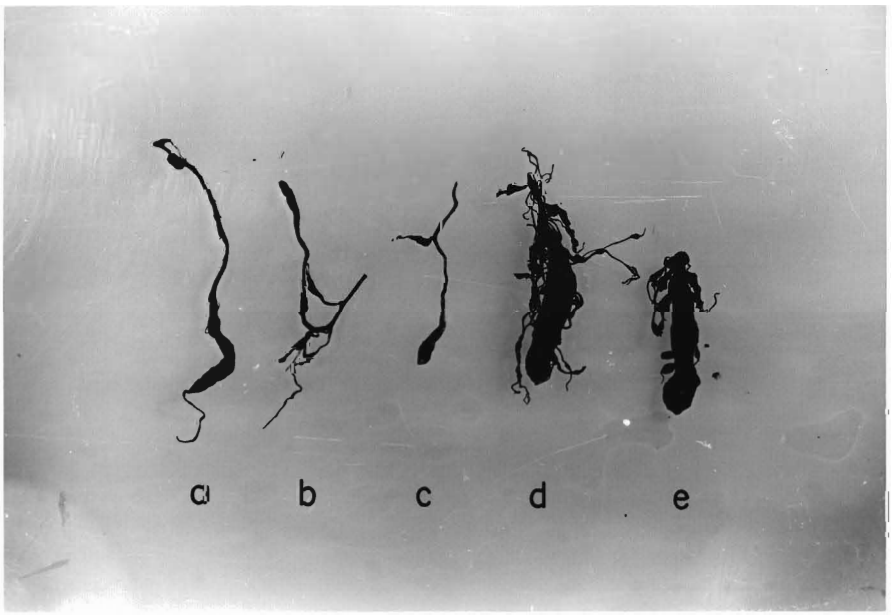


Plate 5. Severely infested root system taken from run 15 (day 30) of experiment 3. Galls are developed to an advanced degree and are continuous along most of the rootlets of the plant. An estimated 80% of this root mass was gall.

Plate 6. A selection of galls at different stages of development. These include juvenile galls associated with early stages of galling (b, c), mature easily visible galls (a) and well developed advanced stage galls (d, e).

PLATE 5

PLATE 6



Roots of resistant plants were exposed to levels of infectious second stage M. incognita larvae of 17,000 and 18,000 per plant in experiments 6 and 4 respectively. Galls were never encountered in any of these roots nor were there any overt signs of lesions (Fig. 38, plate 1).

5-10. Effects of Galling on Ethane and Ethylene Concentrations of Roots

Changes in ethane and ethylene concentrations over the time course of an experiment should give an indication of any effect nematode infestation had on these gases. The extent of galling in any given root was however, subject to some variation so that it could not be assumed that the severity of infestation increased linearly with time. Figure 37 illustrates this case. Consequently the roots of all experimental runs were grouped according to the severity of nematode infestation, against which the concentrations of ethane and ethylene in roots were compared.

Using the percentage gall of total root mass estimates, roots were grouped into four categories: 1 = 0%, 2 = 1-24%, 3 = 25-49%, 4 = 50-100%. Due to the imprecise nature of the estimates, only four categories were chosen to give greater confidence in the accuracy of this classification. For each root gas sample of each run, a percentage difference between control and infested was computed. This amounted to two values per experimental run. The mean of all gas percentage differences for roots within a given gall classification was calculated. These means were then plotted against gall classifications 1-4. A one-way analysis of variance was computed for both ethane and ethylene from experiments 1, 2, 3 and 5.

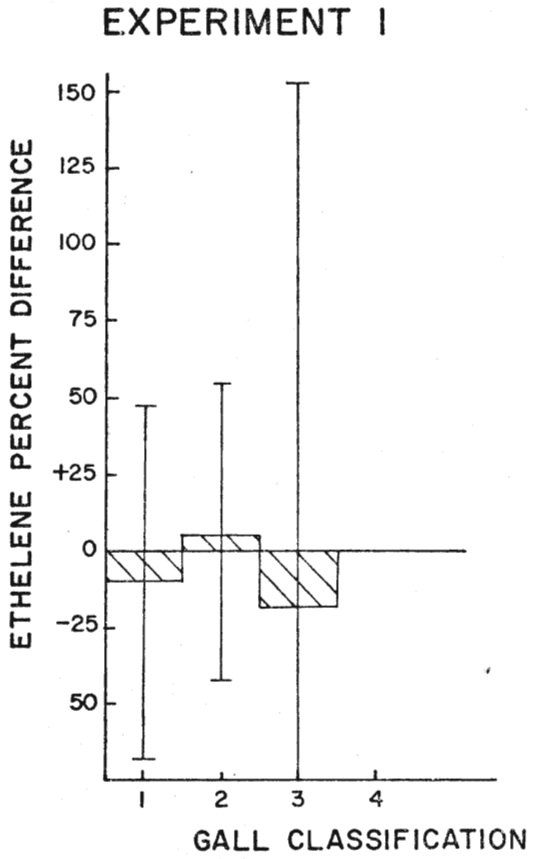
Significant variation between mean percentage differences of gas concentrations from one gall classification to another were uncommon. The only significant variation of these means between gall classification was

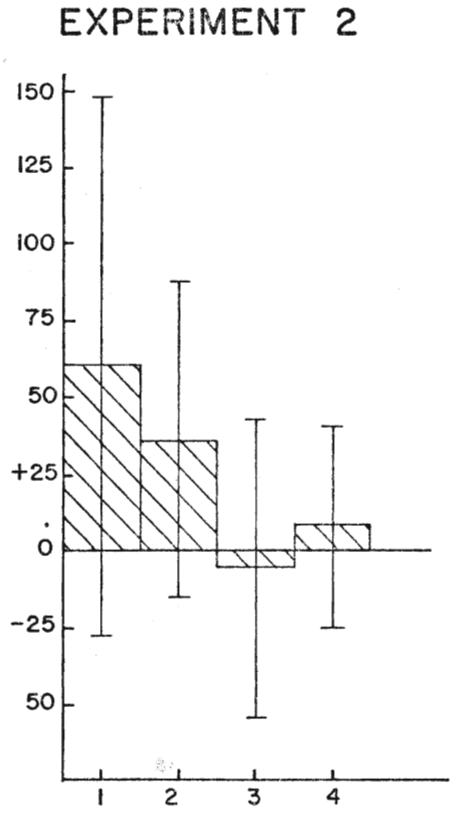
found in experiment 5 ethylene data. Marginal significance was found for ethane data from experiment 5 and ethylene data from experiment 3. The mean ethylene percentage difference of gall classification 2 in experiment 5 notably favoured infested roots (i.e., ethylene concentrations were greater in infested than control roots). Mean percentage differences of the other groups in experiment 5 were less pronounced (Fig. 39). In contrast, group 2 ethylene percentage differences in all other experiments indicated higher concentrations in control roots. Examination of the histograms in Figures 39 and 40 reveals that there was no consistent variation of mean gas percentage differences across gall classifications for all experiments. In most cases, there were no significant variations and where significance (or marginal significance) was found, the pattern of mean gas percentage difference for a given gall classification contrasted with that determined in other experiments. Thus the hypothesis that elevated levels of ethylene (or ethane) would be found with increased galling was not substantiated by these results.

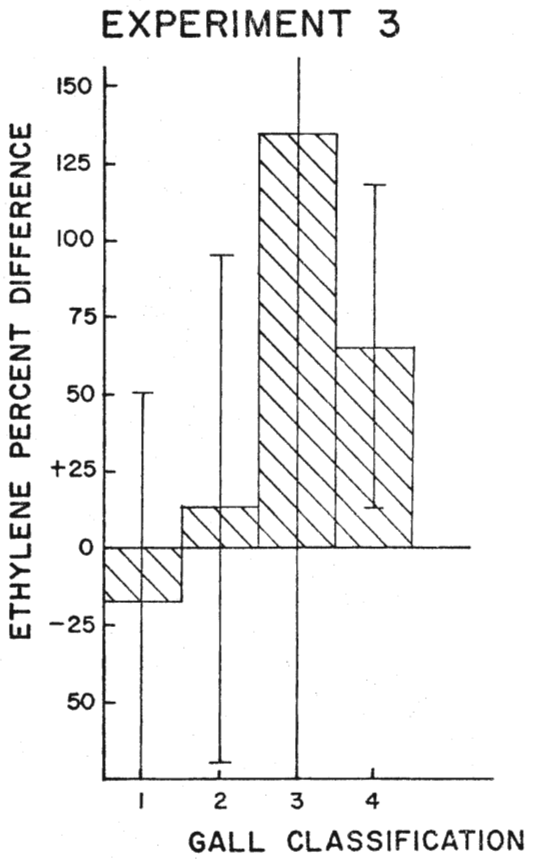
5-11. Rates of Ethylene Production

Various methods have appeared in the literature for the collection of ethylene evolved from plant tissues. The earliest methods involved placing plant tissue into a closed vessel out of which a sample of the atmosphere could be withdrawn with a syringe and analysed via gas chromatography. This technique had the drawback of creating artificial conditions since partial pressure of ethylene could be built up in the atmosphere, pCO_2 would decrease due to photosynthesis and pO_2 would increase via the same process. Within limits the longer the tissue was left in the vessel the

Figure 39. Ethylene percentage differences versus gall classification. The mean percentage difference of gas concentration within each category of gall severity is indicated along with 95% confidence limits. Gall classifications were 1 = 0%, 2 = 1-24%, 3 = 25-49% and 4 = 50-100% of the total root mass estimated to be gall.


EXPERIMENT 1
ETHELENE PERCENT DIFFERENCE
150
125
100
75
50
+25
0
-25
50
1
2
3
4
GALL CLASSIFICATION


EXPERIMENT 2
150
125
100
75
50
+25
0
-25
50
1
2
3
4


EXPERIMENT 3
ETHYLENE PERCENT DIFFERENCE
150
125
100
75
50
+25
0
-25
50
1
2
3
4
GALL CLASSIFICATION

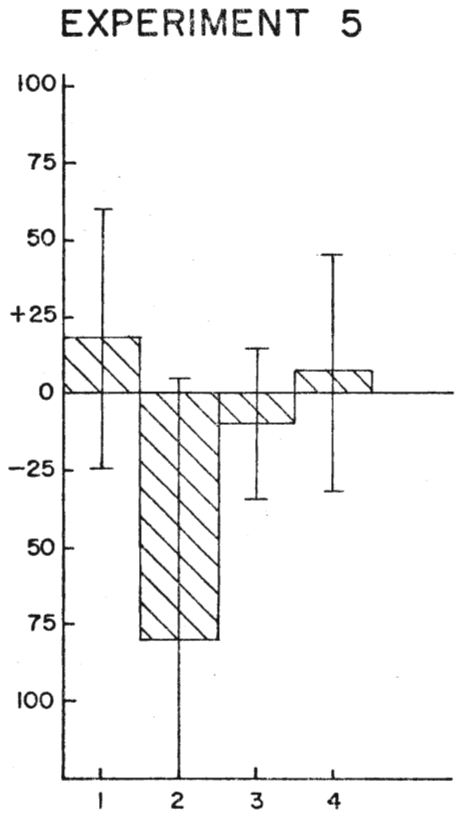
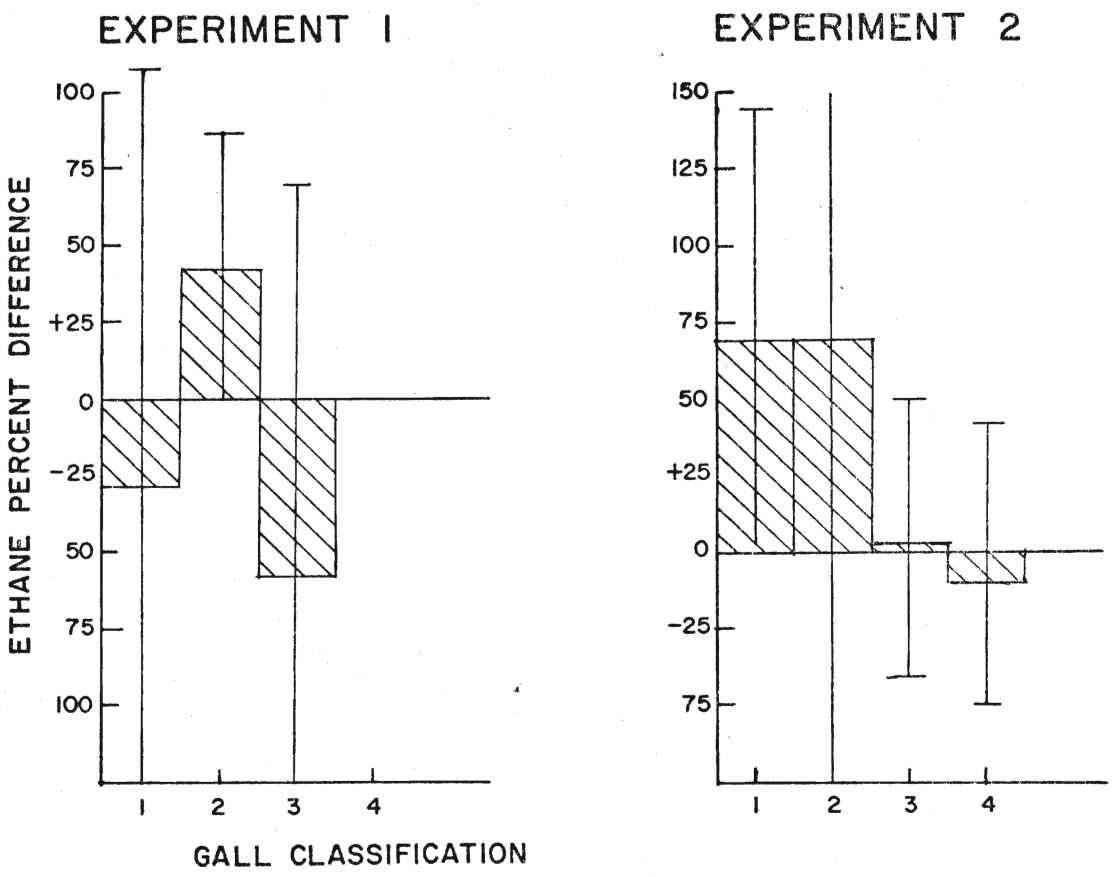
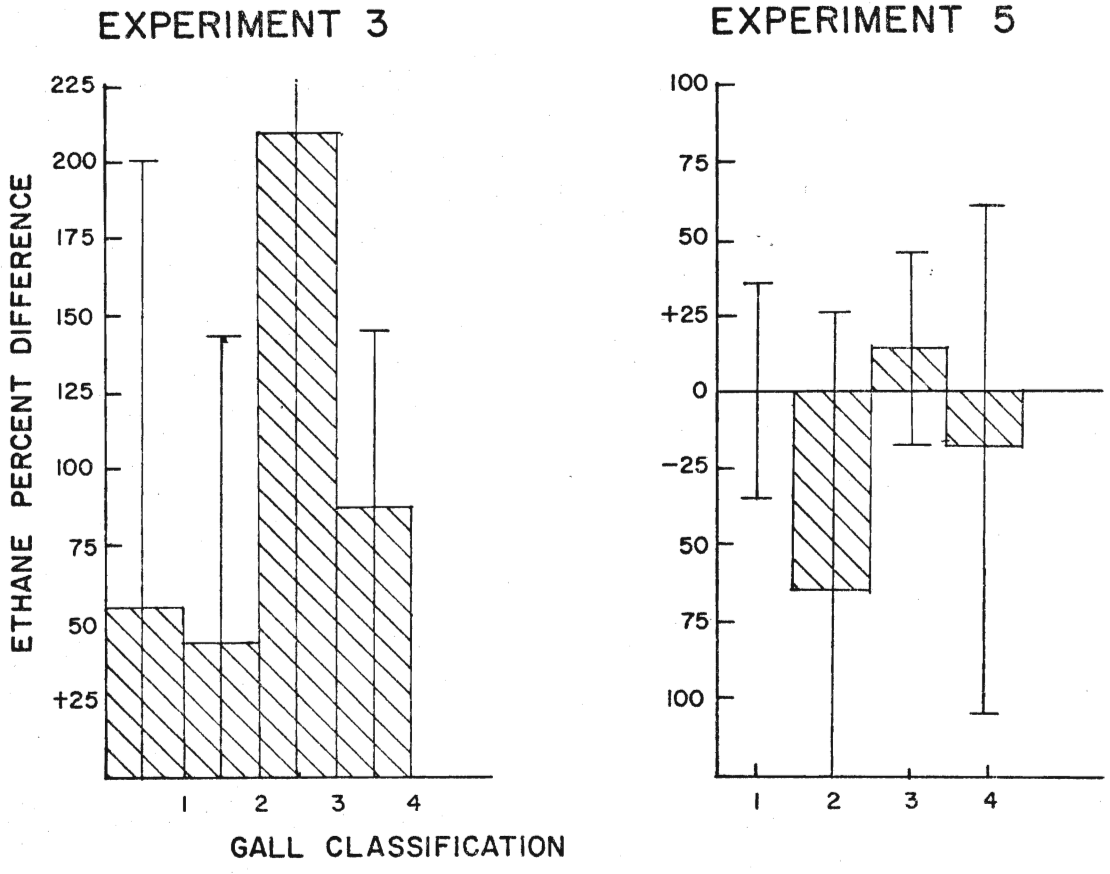

EXPERIMENT 5
100
75
50
+25
0
-25
50
75
100
1
2
3
4

Figure 40. Ethane percentage differences versus gall classification.


EXPERIMENT 1
EXPERIMENT 2
ETHANE PERCENT DIFFERENCE
100
75
50
+25
0
-25
50
75
100
150
125
100
75
50
+25
0
-25
75
1
2
3
4
GALL CLASSIFICATION


EXPERIMENT 3
EXPERIMENT 5
ETHANE PERCENT DIFFERENCE
225
200
175
150
125
100
75
50
+25
100
75
50
+25
0
-25
50
75
100
1
2
3
4
GALL CLASSIFICATION

the greater these effects would be. Most recent publications concerning the rate of ethylene evolution from plants have utilized flow-through systems. These consist of a chamber (usually plexiglas) sealed about a section of plant tissue through which a slow flow of air is passed. Ethylene in the effluent air may be trapped by agents such as liquid nitrogen (Abeles 1973) or silica gel (Phan 1976). Collecting the gases evolved from a small area of plant tissue (i.e., section of stem or a leaf) allowed slow air flows to be used (i.e., 270 ml/minute, Pegg and Cronshaw 1976). The apparatus designed and used in the present investigation was of a large volume which accommodated 4 to 6 mature tomato plants. The purpose was to collect total ethylene evolved from control or infested plants. It was felt that to minimize partial pressure gas changes in the chamber, a relatively fast air flow was needed. A flow of 3300 ml/minute was calculated to theoretically flush out the atmosphere of the chamber every 10 minutes. This flow rate was selected for experimental runs.

Numerous attempts to trap ethylene with a variety of techniques were made. The first technique involved freezing ethylene out of the effluent air by passing the air through a copper loop submerged in liquid nitrogen. Problems were encountered with this method as CO_2 and H_2O were also frozen out in the ethylene trap, thereby plugging it. This was corrected by placing a CO_2 trap (vessel packed with Indicarb 10-20 mesh) and H_2O trap vessel submerged in a dry ice-acetone bath) in series before the liquid nitrogen bath. However, removing the copper loop and attaching it to the gas chromatograph while maintaining liquid N_2 temperatures was a difficult task. Further, upon warming the loop to room temperature to permit the trapped gaseous contents to be flushed through the gas chromatograph, a

pressure build up within the loop resulted in leakage of the gases at the swagelock fittings. This method of trapping ethylene was therefore abandoned.

Copper loops packed with 80 mesh silica gel could not accommodate much more than 'trickle' flows of air to pass through and were thus unsuitable for the experiments.

The loops were also packed with 4 Å molecular sieves which absorb ethylene at room temperature (Friesen and Friesen 1967). To regenerate trapped gases, the molecular sieves were heated to 350°C in molten solder baths while connected to the gas chromatograph. Although the procedure of collecting gases from plants and attaching the loops to the gas chromatograph was easily accomplished with this technique, no consistent ethylene peaks were obtained from numerous trials. It was felt that the absence of consistent ethylene peaks reflected inadequate technique rather than a rate of ethylene production too low to measure.

DISCUSSION

Root Weights, Galling and Nematode Infestation

The effect of Meloidogyne incognita infestation on susceptible and resistant tomato plants was expressed in the relative root weights of control as compared to nematode-treated plants. For most experiments performed, control and nematode-treated root weights were similar over the first few experimental runs. Exceptions to this rule were found in experiments 5 and 6 which will be discussed later. Galls appeared on infested susceptible roots as early as day 4 of experiments 2 and 5, day 6 of experiment 3 and day 11 of experiment 1 (Fig. 37). Once galls had been initiated, they developed fairly rapidly. This phenomena resulted in higher root weights of infested roots. Differences between control and infested root weights increased linearly with time. Since controls were grown under identical conditions, difference in root weight may be attributed to the weight of galls in the roots. This phenomenon was also observed in another laboratory. Olthof and Potter (1977) found greater root weights 8 weeks post-infestation in Meloidogyne hapla infested tomatoes (var. Veebrite) than in controls.

Susceptible plants of experiment 1 showed no significant difference between control and infested root weights even though galls accounted for a maximum estimate of 25% of total root weights (figs. 8, 37). This may have resulted from a combination of factors. Firstly, only 2,000 Meloidogyne incognita larvae were applied per plant in experiment 1. The plants when infested were 46 days old and about 7 cm in height with correspondingly small root systems. Since root systems were minimally developed and low levels of nematode inocula were applied, relatively small numbers of larvae

would have entered the roots. Although galls developed normally on these roots, their effect was masked since plants were at a very active stage of growth and considerable new root growth was added with time (i.e., a 430% increase over 36 days). This growth is indicated by the steep slopes of increasing infested and control root weights with time in Figure 8C.

The absence of any increased root weight in infested plants in experiment 1 despite an estimated 25% galling might have been due to a reduction of overall growth of the root system. Temporary inhibition of mitosis in root meristem tissue has been observed immediately following root-knot nematode penetration (Christie 1936, Thorne 1961). It may be that this occurred in the infested plants of experiment 1. Intuitively it may further be reasoned that such inhibitions would be most pronounced in young 'fragile' plants such as those of experiment 1.

Plants from experiments 2 and 3 were found to have infested root weights increasing over their controls with time (figs. 8, 9). This indicated that considerable galling occurred in the infested plants of these experiments. Nematode-treated plants of experiment 2 were exposed to 10,000 larvae per plant, while those of experiment 3 received 18,000. Plants in experiments 2 and 3 were 95 and 90 days old respectively and had well-developed root systems at infestation. The plants were mature as indicated by flowering prior to infestation. Galls developed more rapidly in experiment 3 than in experiment 2 (fig. 37). Multiple entry through single ports may have occurred (Thorne 1961, Bird 1961). Gall size has been shown to be proportional to the number of nematodes within the gall (Orion and Minz 1969). It is quite possible that more nematodes were present in the galls of experiment 3 than experiment 2 because of the greater number of larvae

applied. More individual galls were apparent on these roots as well, but precise numerical counts could not be made.

Differences in rates of gall development may have been due to seasonal variations. Plants in experiments 3 and 5 for example received approximately equal numbers of larvae, however gall development was considerably more rapid and extensive in experiment 3. Experiment 3 was conducted in summer (July-August) while experiment 5 was conducted in winter (December-January). The shorter days, less intense sunlight and lower temperatures of winter affected plant growth despite greenhouse temperature control and a 16 hour photoperiod provided by fluorescent lights. Nematode development is sensitive to environmental conditions (Crofton 1966, Endo 1975) and gall formation has been found to coincide with the development and growth of the nematode in the roots (Owens and Specht 1964; Bird 1967, Orion 1973). It may be that seasonal differences affected nematode development and hence gall formation in these experiments.

Control root weights of experiment 5 plants decreased while infested roots maintained a constant weight over the experimental time course (fig. 12). The decrease in root weights of controls may have been due to senescence. Plants for experiment 5 were 125 days old at infestation, their root systems had filled the 10 cm square pots to a large degree and the experiment was conducted in the winter months. Symptoms of senescence were visible in the yellowing and abscission of leaves. It is possible that the decrease in root weight observed for control plants was due to degeneration of roots. Infested roots did not decrease in weight. This was due to the added weight of galls which compensated for degeneration of roots.

Resistant plants of experiment 4 had similar control and nematode treated root weights, a result consistent with the observation that no galls ever formed on resistant roots. This result further supports the suggestion that higher root weight increases of susceptible infested plants was due to galling.

Both nematode treated and control root weights of resistant plants in experiment 6 were observed to decrease presumably due to senescence and degeneration (fig. 13). The rate of this decrease was similar for both control and nematode-treated roots as determined by linear regression analysis of the differences. However, nematode-treated roots were consistently greater in weight than control roots. It is suggested that this was due to a difference in growth conditions of nematode-treated and control plants since no galls were ever observed on these roots. Figure 4 shows the arrangement of plants for experiments 5 and 6 (run concurrently) as compared to experiments 1 through 4. It can be seen that while each bench for the first four experiments contained 1/2 infested and 1/2 control plants, experiments 5 and 6 had separate benches for nematode-treated and control plants. In these experiments nematode-treated plants were located centrally and controls peripherally under the fluorescent lights. The shorter days of winter and less direct sunlight may have increased the importance of the bank of fluorescent lights on plant growth. Centrally located plants were directly under the lights while peripherally placed ones would have received less fluorescent light. The nematode treated plants of experiments 5 and 6 were grown in this location before, during and after infestation without any change in position. It is proposed then that the nematode treated plants of experiment 6 had already established healthier root systems than controls and that infestation had no

effect on root weight, the difference observed being due to the difference in light available to these two groups of plants. The experimental design here could have been improved by randomizing the plants prior to infestation as they were in previous experiments.

Effects of Nematode Infestation on Stem Heights

Both nematode infestation and ethylene application can result in stunting of stem height (Orion and Minz 1969, Brueske and Bergeson 1972, Abeles 1973, Orion 1973). Susceptible infested plants of most experiments showed some stunting of stem growth (Results Section 5-1). Stem heights of control plants originally equal to those of infested plants progressively increased in length over their nematode infested counterparts in experiment 2 (fig. 9). This significant difference increased in a linear manner with time, indicating that as gall development progressed infested stems elongated at a slower rate than non-infested plants. No significant differences between experiment 1 infested and control stem heights was found. However, there was a detectable trend from day 18 onward where infested stem heights were less than controls (fig. 8). The absence of a significant difference here was probably due to the low levels of nematode inocula applied and masking of the effects of infestation due to rapid plant growth as previously discussed.

Stem heights of experiment 3 control plants were marginally significantly greater than infested stem heights, however they were increasing significantly in this way if the last atypical value (run 15) was rejected (fig. 10). It is surprising that this difference was not greater (i.e., more significant) since nematode treated plants of experiment 3 were the most severely infested of all experiments. It was noted that control plants of this experiment did

not increase in stem height to a large degree with time (less than experiment 2). Infested plants had virtually stopped stem elongation from day 9 onwards but since control plants were not elongating rapidly the difference between these was slow to develop. At any rate evidence for the stunting of stem elongation subsequent to infestation was present in this experiment.

No significant differences between control and infested susceptible plants in experiment 5 or resistant plants in experiment 6 were found. As discussed previously the plants in the two experiments did not show signs of active growth. Since no stem elongation was occurring in these plants (figs. 12, 13), no effect of nematode infestation on this growth phenomenon could be detected.

Resistant nematode-treated plants in experiment 4 had significantly greater stem heights than their controls. There is no obvious explanation for this result and it could, perhaps, be due to random chance.

Effects of Nematode Infestation on Top Weights

All infested susceptible plants showed significantly greater top weights than their controls except in experiment 2 where this difference was significant at 80% confidence only. It is known that growth responses regulated by ethylene in healthy plants are observed in root-knot nematode parasitized plants (Orion and Minz 1969). The present investigation reconfirmed that nematode infestation had a stunting effect on stem elongation, a result consistent with stimulated ethylene production in the stems of these plants. Concurrent with ethylene inhibited stem elongation is a promotion of lateral expansion (swelling) of the stem tissue. This is due to a reoriented deposition of cellulose microfibrils in the cell walls (Eisinger and Burg 1972). It is possible that heavier top weights of infested plants in

experiments 1, 2 and 3 resulted from increased ethylene production in the stems of nematode infested plants.

Resistant plants in experiment 4 showed no significant difference between nematode-treated and control top weights. This result supports the previous suggestion.

Susceptible plants in experiment 5 and resistant plants in experiment 6 both exhibited significantly greater top weights in nematode-treated plants. Again this can be attributed to an imbalance of the growth conditions of nematode-treated and control plants in these experiments. As was discussed previously, nematode treated plants in these two experiments were more fully developed at infestation than control. Thus, they had heavier top weights at the start of the experiments and since plants in these experiments did not show signs of active growth during the experiment, this difference in top weight remained constant.

Veebrite var. tomatoes subjected to moderate doses of Meloidogyne hapla (i.e., 1840 larvae/kg soil) had greater top weights than controls 8 weeks post-infestation (Olthof and Potter 1977). This result is also consistent with a possible stimulation of ethylene production in stems of infested plants.

Summary of the Effects of Nematode Infestation on Plant Growth

Analysis of the growth of nematode-treated and non-treated plants provided an estimate of the pathogenicity resulting from nematode infestation. This was most obvious in the measurement of root weights where dramatic increases often occurred due to galling induced by the nematodes. Results also indicated that stem elongation was inhibited and top weights increased

by nematode infestations of susceptible plants. No alterations in plant growth of resistant plants were found after nematode-treatment. Alterations in the growth of susceptible plants infested with Meloidogyne incognita are consistent with the possibility that increased ethylene production occurred (i.e., stress ethylene) in the stems of these plants.

Ethylene Levels in Resistant Plants

Meloidogyne incognita larvae penetrate the roots of resistant tomato plants as easily as susceptible ones (Riggs and Winstead 1959). Resistance of Anahu var. tomatoes to this pathogen is controlled by a single incompletely dominant gene (M_i) (Winstead and Riggs 1963), the nature of which is expressed in a hypersensitive host response (HR) (Paulson and Webster 1972, Webster and Paulson 1972). Only cells in the immediate vicinity of the parasite undergo the HR and there is evidence that it occurs only in cells on which the parasite has fed (Paulson and Webster 1972). Thus, it can be assumed in the present study that M. incognita larvae penetrated the resistant roots but were unable to develop since they could not induce syncytia to form.

As indicated in the introduction, published data suggests that ethylene is involved in the mechanism of resistance to certain fungal and bacterial phytopathogens (Clare et al. 1966, Sequira 1973). It has been proposed that ethylene acts to stimulate the activities of tyrosine and phenylalanine ammonia lysases (Giebel 1973) and peroxidases (Riov et al. 1969, Gahagan et al. 1968, Ridge and Osborne 1970). The products of these enzymes all affect the activity of IAA-oxidase. It has been postulated that increased IAA-oxidase activity is the key factor in the mechanism of resistance (Wilski and Giebel 1972).

Comparison of ethylene concentrations from susceptible and resistant roots of control plants indicated possible differences between ethylene concentrations for these two phenotypes. Root concentrations of ethylene from susceptible plants were comparable across all experiments (Results Section 5-6). Ethylene concentrations of resistant control plants of experiment 6 (fig. 29) were similar in range and generally varied in parallel with ethylene concentrations of experiment 5 susceptible control plants (fig. 27). In addition, no difference between ethylene concentrations of experiment 4 resistant plants and experiment 3 susceptible plants was found (fig. 23 and 25). It is concluded from this work that endogenous ethylene levels of Anahu var resistant tomatoes are not different from Vendor var. susceptible tomatoes. Similarly, Pegg and Cronshaw (1976) determined that near isogenic lines of tomato plants (var. Craigella) resistant and susceptible to the fungus Verticillium albo-atrum had similar rates of endogenous ethylene production prior to infestation.

Internal ethylene concentrations of a tissue vary in direct proportion with the rate of ethylene produced by the tissue so that the magnitude of the gradient between internal and external ethylene levels remains constant (Abeles 1973). For example, a tissue with an internal ethylene concentration of 1.0 ppm in ethylene-free air has 1.2 ppm ethylene when 0.2 ppm ethylene is added to the surrounding atmosphere. The gradient between internal and external ethylene levels remains constant at 1.0 ppm. The ratio between internal ethylene levels and the rate of production of ethylene can therefore be expressed as a conversion constant equal to ppm ethylene in the tissue per ml ethylene produced per kg fresh weight per hour. Kang and Ray (1969) found equal conversion constants for ethylene and carbon dioxide of 0.42

ppm/ml/kg/h in bean hypocotyl tissue. Chadwick and Burg (1967) found a conversion constant for ethylene from pea roots of 0.3 ppm/ml/kg/h. Thus measurements of the internal ethylene concentration of a tissue reflect the rate of production of ethylene by that tissue.

It may be reasoned that if ethylene action is involved in Anahu resistance to M. incognita, increases in ethylene production must occur after infestation since endogenous production rates were similar for resistant and susceptible plants. This has been observed in resistant responses of host plants to other pathogens (Weise and De Vay 1970, Shain and Hillis 1972, Sequira 1973). Shain and Hillis (1972) investigated ethylene production in the gymnosperm Pinus radiata from tissue surrounding lesions made by oviposition of the wood wasp Sirex noctilo. At oviposition Sirex also deposits arthrospores of the decay fungus Amylosterum areolatum into the lesion. Two varieties of Pinus radiata, one resistant to this fungus and the other susceptible, were investigated for rates of ethylene production after Sirex oviposition. An increase in ethylene production from 2.5 ml/g dry wood/h in control resistant tissue to 42.3 ml/g dry wood/h after oviposition was found. This represented an increase of 17 fold. The greatest single increase in root-knot nematode treated resistant tomato roots was 175% that of resistant control roots. In addition, no significant differences were detected between nematode treated and control resistant plants. These results indicate that ethylene production as measured by internal root concentrations was not stimulated with the resistant response. Similarly, rates of ethylene production in tomato plants resistant to Verticillium albo-atrum were not altered subsequent to inoculation with this fungus (Pegg and Cronshaw 1976).

Ethylene Levels in Susceptible Plants

In no experiments were significantly greater ethylene concentrations obtained from roots of infested plants. In fact, ethylene concentrations were often greater in control than in infested plants. Experiment 3 was determined to have mean control root ethylene concentrations 47% greater than mean infested root ethylene concentrations (Results Section 5-6). This significant difference between control and infested root ethylene concentrations increased with time. Experiment 2 had ethylene concentrations of control roots 18% greater than infested roots which was a marginally significant difference. No significant differences were found for control and infested root ethylene concentrations of experiments 1 and 5. In contrast to the hypothesis presented in the introduction, the present work indicates that not only do ethylene concentrations not increase subsequent to nematode infestation and gall development, but actually appear to decrease.

Orion and Minz (1969) reported that Meloidogyne javanica induced galls on tomato roots increased proportionally with the amount of exogenous ethrel (ethylene precursor) applied. Later work confirmed this response of Fortcs var. tomato with M. incognita induced galls and ethrel application (Orion and Hoekstra 1974). It was suggested that increased ethylene levels were involved in the pathogenicity, specifically in the development of galls (Orion 1973). Results of experiment 3 indicate however that as the galling response increased the concentration of ethylene per gram root decreased.

Further analysis examined the relationship of ethylene root concentrations to the extent of galling. Analysis of variance calculations were performed on ethylene concentrations grouped into four gall classifications (Results Section 5-10). No consistent variations in ethylene concentration with

extent of gall formation was detected by this procedure.

Comparisons were then made between ethylene concentrations and root weights as a different indicator of the relationship between extent of galling and tomato root ethylene concentrations (Results Section 5-8). Linear regression analysis of nanograms ethylene per gram root versus root weight for all susceptible plants (experiments 1, 2, 3 and 5) revealed that in most cases there was a proportional increase in amount of ethylene with increasing root weight. However, the infested plants of experiment 3 which were the most severely galled of all experiments, did not show this relationship and total ethylene levels did not keep pace with increases in root weight. The same result was found for experiment 1 infested plants but was significant at only the 90% level. Experiments 1, 2, 3 and 5 susceptible control plants were determined to have proportionally increased ethylene levels with increased root weights. These results suggest that increases in ethylene levels of infested tissues did not keep pace with increasing root weight. This suggests that on a per gram weight basis, gall tissue contains less ethylene than normal root tissue.

That this trend was not found in experiments 2 and 5 is readily explainable. It has been noted that the roots of infested plants in experiment 5 did not increase in weight over the experimental time course while controls actually decreased (Results Section 5-1). Since root weights did not change much the relationship between ethylene levels and changing root weights could not be determined. Ethylene concentrations of experiment 2 infested and control plants increased dramatically at day 14 assuming a new elevated range of concentrations for the remainder of the experiment (fig. 21). This abrupt increase at day 15 overshadowed the effects increases in root weight had on ethylene levels.

A limited number of studies have investigated the relationship of ethylene production in plants infested by pathogens on a time course basis. None of these involved plant parasitic nematodes. One of the more rigorous investigations of this sort was that recently conducted by Pegg and Cronshaw (1976). They investigated rates of ethylene production from stem internodes and leaves of tomato varieties susceptible and resistant to a strain (T179) of the fungus Verticillium albo-atrum. Elevated ethylene production was found in stems and leaves after infestation and this rise in ethylene production either coincided with or was detected shortly after symptom development (i.e., epinasty, chlorosis, necrosis, adventitious roots, loss of turgor). Ethylene production in the first stem internode increased to a maximum of 12.83 nl/gram dry weight/h, 12 days after fungal infection. This represented a 44-fold increase in the rate of ethylene production over the rate prior to infestation. The rate of ethylene production by the second leaf of susceptible tomato plants increased from about 2 nl/g dry weight/h 6 days post-infestation to 24 nl/g dry weight/h 9 days post-infestation, an increase of 12 times the former production rate. In both instances (in stem and leaf) increased ethylene production was detected shortly after visible pathogenic symptoms had appeared. The authors interpreted this to indicate that the increase in ethylene production was stress ethylene resulting from the pathological conditions and that ethylene itself was not acting as a phytotoxin and was not involved in the mechanism of infestation.

Other investigators have found similar dramatic increases in ethylene production-associated with disease. Olsen et al. (1970) found an increase in internal ethylene concentrations of lemons and limes infected with Stubborn virus of about 110 fold (i.e., control levels 0.01 ppm ethylene,

virus-infected levels 1.1 ppm ethylene). Talboys (1972) incubated stem segments of hops infected with V. albo-atrum or V. dahlia and collected the ethylene evolved. Results indicated an increase in ethylene production up to 33 x control rates in infected stem segments. Weise and De Vay (1970) however reported only 2 and 5 fold increases in rate of ethylene production in cotton plants infected with non-defoliating and defoliating strains of V. albo-atrum respectively. Their technique, in which plants were enclosed in polyethylene bags or jars for the collection of gases has been criticized (Pegg and Cronshaw 1976).

These results are uniform in that rates of host plant ethylene production increased subsequent to pathogenic infection. The magnitude of these increases were generally in the range of 10 to 100 times. If changes in ethylene levels are involved in the relationship between M. incognita and tomato plants, similar large changes in ethylene may reasonably be expected. However, in the present study the greatest difference in ethylene levels of M. incognita infested versus control roots was a 2½ fold increase 36 days post-infestation in only one experiment (experiment 1, run 15). Values of ethylene levels of infested roots for the greatest majority of determinations ranged with ½ to 2 times ethylene levels of control plants. Increases in ethylene concentrations of M. incognita infested versus non-infested tomato roots in the order of 10 to 100 fold as found by other researchers in similar studies were never encountered.

The hypothesis in the literature that ethylene is involved in the galling response of host plants to root-knot nematodes (Orion and Minz 1969, Orion 1973) was not supported by the present study. Ethylene concentrations on a per gram root weight basis either decreased significantly as root

weights increased due to galling, or showed no significant difference where the increase in root weight of galled plants was not large. Orion and Minz (1969) based their proposal on the fact that ethrel application increased gall size of tomato seedlings (var. Hossen Eilon) infested with Meloidogyne javanica, However, a later publication revealed that ethrel treatment did not affect tomato galls induced by M. hapla. Further, although root-knot nematode induced galls of cauliflower, cucumber and calendula increased in size subsequent to ethrel application, galls on okra, carrot and wheat did not (Orion 1973). In another publication similar discrepancies arose. Orion and Hoestra (1974) determined that galls on Fortos var. tomatoes induced by either M. incognita or M. javanica increased in size with ethrel application. However, galls ofMoneymaker var. tomato were unaffected. These results are perplexing. It is difficult to believe in the involvement of ethylene in gall formation when treatment with the ethylene procursor ethrel resulted in increased gall size in one tomato variety but not in another when both were infested with the same nematode (M. incognita). Although this hypothesis was proposed in 1969 (Orion and Minz), it was restated in 1973 (Orion) and not reconsidered in light of the variety of host-parasite responses to ethrel reported in 1973 (Orion and Hoestra).

The explanation given for ethylene action in gall formation as detailed by these researchers is also questionable. It was suggested (Orion and Minz 1969) that ethylene caused proliferation of parenchyma tissue of galls by a mechanism analogous to that which causes swelling in etiolated pea stems (Burg and Burg 1968). However, the swelling of pea stem tissue results from increases in cell size, whereas the increase in gall size was reported to involve an increase in mitosis of parenchyma cells. It is my contention,

as supported by the experiments reported in this investigation, that ethylene does not play a significant role in gall formation. It may be that applications of the high ethrel doses used by Orion and Minz (1969) resulted in a pathological condition in the plants due to the release of toxic levels of ethylene. As a result the metabolism of some hosts was altered such that galling increased while in others it did not. It seems likely that results obtained by Orion and Minz resulted from ethylene concentrations that were not physiological.

Ethane Levels in Susceptible and Resistant Plants

Recently Elstner and Konz (1976) reported on ethylene and ethane production in sugar beets. Leaf discs were point frozen with a 3 mm diameter stainless steel rod kept at liquid nitrogen temperatures. Production of both gases increased linearly as greater percentages of the leaf disc surface areas were frozen. Ethylene production peaked when 50% of the leaf disc areas were frozen and decreased linearly as greater proportions were frozen. With 100% of the leaf disc area frozen, ethylene production was negligible. Ethane production however increased linearly to a maximum rate with 100% frozen. It was suggested that ethylene production increased in the cells immediately surrounding the damaged cells. This would account for the maximum production rate observed when 50% of the surface area of the leaf discs were frozen. Freezing greater proportions resulted in decreased ethylene productions. The authors speculated that intact cellular compartmentalization was necessary for ethylene but not ethane biosynthesis since ethane production was maximum when the leaf discs were entirely (100%) frozen. This result was substantiated by a further test in which sugar

beet leaf discs were pressed with a plastic piston and ethane and ethylene productions measured (Elstner and Konz 1976). While the rate of ethylene production decreased sharply after pressing, ethane production increased sharply. The authors proposed that ethane and ethylene are produced via two different biosynthetic pathways.

It has been well documented that tissues of higher plants stop producing ethylene after homogenization while ethane production continues (Burg and Burg 1961, Lieberman and Mapson 1962, Curtis 1969). Apple sub-cellular particulate preparations evolved ethane only when a source of unsaturated fatty acid was present in the system (Lieberman and Mapson 1962). Recently John and Curtis (1977) isolated and characterized linolenic acid as the specific unsaturated fatty acid required for ethane production in tissue homogenates of Phaseolus vulgaris. Additionally a high molecular weight soluble enzyme was determined to be involved in ethane biosynthesis. They suggest that linolenic acid is the precursor of ethane biosynthesis in P. vulgaris. John and Curtis (1977) also observed that oat root tissue homogenates supplemented with linolenic acid demonstrated an 8 fold increase in ethylene production. Ethane production also increased in these preparations in response to linolenic acid, a result which is consistent with the proposal that linolenic acid is the precursor for ethane biosynthesis. Since linolenic acid increased both ethane and ethylene production in this system, it may be that some common factor is involved in the biosynthesis of both gases. This interpretation is in contradiction to that proposed by Elstner and Konz (1976).

Root gases in the experiments reported here contained both ethane and ethylene. Ethane concentrations were determined since controversy existed in the literature concerning the relationship between ethane and ethylene

biosynthesis. Without exception, every root gas sample analysed had a greater concentration of ethane than ethylene. This result is contrary to the findings of Elstner and Konz (1976)who found greater ethylene than ethane production in sugar beet leaf discs.

The apparent parallel variations of ethane and ethylene with time in most experiments has been documented in the results (Section 5-6). Statistical analysis of ethane and ethylene percentage differences between control and infested plants supported this observation. In every experiment where a significant difference in ethylene levels was found a similar significant difference for ethane was found. For example, ethylene concentrations from the roots of plants in experiment 3 (susceptible) were significantly greater in control than infested plants. This same result was found for ethane. Where there was no significant difference in ethylene concentration there was also no significant difference in ethane concentrations. Only one exception to this pattern was found and that was a significantly greater mean ethane concentration from control resistant plants in experiment 6. As previously discussed ethylene concentrations of susceptible plants were either unaffected or decreased relative to controls after root-knot nematode infestation. Since ethane concentrations were affected in the same way as ethylene concentrations, it appears that nematode infestation affected the synthesis of these two gases in a common manner. These results would seem to corroborate the results obtained by John and Curtis (1976).

Ethane/Ethylene Ratios

In order to investigate further the relationship of these gases, ratios of ethane concentrations over ethylene concentrations were calculated. In 4 out of 6 experiments, ethane/ethylene ratios increased significantly with time for both control and infested plants (experiments 3-6). Ratios of experiment 1 showed no tendency to increase or decrease with time and were scattered over a wide range of values. Ratios from plants in experiment 2 significantly decreased with time (figs. 31-36).

It is known that ethane production in many higher plant tissues increases after homogenization (Lieberman and Mapson 1962). Mechanical damage of sugar beet leaf tissues also resulted in increased ethane production (Elstner and Konz 1976). In the latter case, greatest ethane production rates occurred in the most severely damaged tissues. It appears from these results that ethane production may be associated with degeneration of dead or dying plant cells. Results of experiments reported here may be consistent with this suggestion.

The senescent condition of plants in experiments 5 and 6 has been discussed. Symptoms of this condition were seen in decreasing root weights of control plants with time, the absence of stem elongation and a decrease of top weights with time (fig. 13). Root weights were decreasing with time presumably due to degeneration associated with senescence. It may be that increases in ethane concentration were a direct result of this process. Ethylene concentrations did not demonstrate a concurrent increase. Thus, increasing ethane/ethylene ratios with time for experiments 5 and 6 were observed.

Plants in experiments 3 and 4 were 90 days old at infestation as compared to 125 days old in experiments 5 and 6. These plants were grown in spring

and summer months, grew rapidly and were mature at infestation (see Results Section 5-1, fig. 10-11). It is likely that the aging process was proceding in these plants. Increased ethane/ethylene ratios with time in these experiments perhaps indicate increased ethane production as a result of increases in dead and dying cells associated with aging.

Plants in experiment 1 were young (46 days old at infestation) and rapidly growing (Results Section 5-1, fig. 8). Presumably dead and dying cells were at a minimum in these plants. The absence of increasing ethane/ethylene ratios with time in this experiment therefore is consistent with the hypothesis that increases in ethane production resulted from increases in dead and dying cells.

Results from experiment 2 were anomolous. A dramatic increase in ethylene concentrations at day 14 and onwards was observed. These increases were 5-6 times former levels. Although increases were also observed for ethane concentrations, they were to a lesser extent (i.e., 2-3 times former levels). This resulted in significantly decreasing ethane/ethylene ratios with time. There is no apparent explanation for the observed dramatic increases of ethylene and ethane concentrations.

In general the results obtained upon investigation of ethane/ethylene ratios are consistent with the suggestion made by Elstner and Konz (1976) that ethane production rates reflect the integrity of a tissue. It may be that ethane is anatural product of decay processes in degenerating plant cells.

SUMMARY

1. No significant difference was found between endogenous root ethylene levels of root-knot nematode resistant (var. Anahu) and susceptible (var. Vendor) tomato plants.
2. No significant increase in ethylene levels was detected in roots of susceptible (var. Vendor) tomato plants after infestation with M. incognita. This result does not support the hypothesis in the literature that increased ethylene production accompanies gall formation.
3. No significant changes in ethylene levels in roots of resistant (var. Anahu) tomato plants were detected after exposure to large numbers of M. incognita. This result suggests that ethylene is not involved in the mechanism of this resistance.
4. Root ethylene concentrations decreased in susceptible plants after M. incognita infestation in two experiments (experiments 2 and 3). Root ethane concentrations also decreased in these experiments and the level of significance of this decrease was the same for both gases. This result appears to substantiate the suggestion that the processes of ethane and ethylene biosynthesis may have some components in common.
5. Ethane/ethylene ratios increased significantly with time in 4 of 6 experiments (experiments 3 to 6). The possibility that increases in ethane production were due to an increase of degenerating tissue is discussed.
6. Results indicated that M. incognita infestation of susceptible (var. Vendor) tomato plants influenced the growth of these plants such that root weights increased, stem heights were reduced and top weights increased.
7. Growth of resistant (var. Anahu) tomato plants was unaffected by exposure to large numbers of M. incognita. No galls were ever detected on roots of resistant plants.

7. LITERATURE CITED

1. Abeles, F.B. 1973. Ethylene in Plant Biology. Academic Press, New York.

2. Akitt, D. B. 1975. The Histopathology of Root-Knot Nematode (Meloidogyne incognita) Infection on Vicia faba. B.Sc. Honours Thesis, Trent University, Peterborough.

3. Anonymous. 1973. Statistics Engineering General Library. Wang Laboratories Inc.

4. Apelbaum, A. and Burg, S.P. 1972a. Effect of Ethylene on Cell Division and Deoxyribonucleic Acid Synthesis in Pisum sativum. Pl. Physiol. 50: 117-24.

5. _____ 1972b. Effects of Ethylene and 2,4-Dichlorophenoxyacetic acid on Cellular Expansion in Pisum sativum. Pl. Physiol. 50: 125-31.

6. Apelbaum, A., Fisher, J.B. and Burg, S.P. 1972. Effect of Ethylene on Cellular Differentiation in Etiolated Pea Seedlings. Amer. J. Bot. 59: 697-705.

7. Balasubramanian, M. and Rangaswami, G. 1962. Presence of Indole Compounds in Nematode Galls. Nature 194: 774-5.

8. Baur, A.H., Yang, S.F., Pratt, H.K. and Biale, J. 1971. Ethylene Biosynthesis in Fruit Tissues. Pl. Physiol. 47: 696-9.

9. Beyer, E.M. 1972. Mechanism of Ethylene Action. Pl. Physiol. 49: 672-5.

10. Beyer, E.M. and Morgan, P.W. 1970. A Method For Determining the Concentration of Ethylene in the Gas Phase of Vegetative Plant Tissue. Pl. Physiol. 46: 352-4.

11. Bidwell, R.G.S. 1974. Plant Physiology, Macmillan Publishing Co. Inc., New York.

12. Bird, A.F. 1961. The Ultrastructure and Histochemistry of a Nematode Induced Giant Cell. J. Cell. Biol. 11: 701-5.

13. _____ 1962. The Inducement of Giant Cells by Meloidogyne javanica Nematologica 8: 1-10.

14. Bird, A.F. 1966. Some Observations on Exudates from Meloidogyne Larvae. Nematologica 12: 471-82.

15. _____ 1967. Changes Associated with Parasitism in Nematodes I. Morphology and Physiology of the Pre-parasitic and Parasitic Larvae of Meloidogyne javanica. J. Parasitol. 53: 768-76.

16. _____ 1968. Changes Associated with Parasitism in Nematodes. IV. Cytochemical Studies on the Ampulla of the Dorsal Eosophageal Gland of Meloidogyne javanica and on Exudations from the Buccal Stylet. J. Parasitol. 54: 879-90.

17. _____ Changes Associated with Parasitism in Nematodes. V. Ultrastructure of the Stylet Exudation and Dorsal Eosophageal Gland Contents of Female Meloidogyne javanica. J. Parasitol. 55: 337-45.

18. Bird, A.F. 1972. Quantitative Studies on the Growth of Syncytia Induced in Plants by Root-Knot Nematodes. Int. J. Parasitol. 2: 157-70.

19. _____ 1973. Observations on Chromosomes and Nucleoli in Syncytia Induced by Meloidogyne javanica. Physiol. Plant Pathol. 3: 387-91.

20. _____ 1974. Plant Response to Root-Knot Nematode. Ann. Review of Phytopathol. 12: 69-85.

21. Bliss, C. I. 1967. Statistics in Biology. Vol. I. McGraw-Hill Book Co., Toronto.

22. Brueske, C. H. and Bergeson, G. B. 1972. Investigation of Growth Hormones in Xylem Exudate and Root Tissue of Tomato Infested with Root-Knot Nematode. J. Exp. Bot. 23: 14-22.

23. Brueske, C. H. and Dropkin, V. H. 1973. Free Phenols and Root Necrosis in Nematex Tomato Infected with the Root-Knot Nematode. Phytopathology 63: 329-34.

24. Burg, S. P. 1973. Ethylene in Plant Growth. Proc. Nat. Acad. Sci. U. S. A. 70: 591-7.

25. Burg, S.P. and Burg, E. A. 1961. Ethylene Evolution and Sub-Cellular Particles. Nature 191: 967-969.

26. Burg, S. P. and Burg, E. A. 1966a. The Interaction between Auxin and Ethylene and its Role in Plant Growth. Proc. Nat. Acad. Sci. U.S.A. 55: 262-9.

27. _____ 1966b. Fruit Storage at Subatmospheric Pressures. Science 153: 314-15.

28. _____ 1967. Molecular Requirements for the Biological Activity of Ethylene. Pl. Physiol. 42: 144-52.

29. _____ 1968. Ethylene Formation in Pea Seedlings; Its Relations to the Inhibition of Bud Growth Caused by Indole-3-Acetic Acid. Pl. Physiol. 43: 1069-74.

30. Burg, S. P. and Clagett, C. O. 1967. Conversion of Methionine to Ethylene in Vegetative Tissue and Fruits. Biochem. Biophys. Res. Commun. 27: 125-30.

31. Campbell, R. C. 1967. Statistics for Biologists. Cambridge University Press, Cambridge.

32. Chadwick, A. V. and Burg, S. P. 1967. An Explanation of the Inhibition of Root Growth Caused by Indole-3-acetic Acid. Pl. Physiol. 42: 415-20.

33. _____ 1970. Regulation of Root Growth by Auxin-Ethylene Interaction. Pl. Physiol. 45: 192-200.

34. Change, D. C. N., Campbell, W. F. and Griffin, G. E. 1973. Ultrastructural Changes Induced by Stem Nematodes in Hypocotyl Tissue of Alfalfa. J. Nematol. 5: 165-73.

35. Christie, J. R. 1936. Development of Root-Knot Nematode Galls. Phytophathol. 26: 1-22.

36. Clare, B., Weber, D. J. and Stahmann, M. A. 1966. Peroxidase and Resistance to Cerotocystis in Sweet Potato Increased by Volatile Materials. Science 153: 62-3.

37. Cleland, R. 1967. Inhibitory Effect of Cell Elongation in Avena Coleoptile by Hydroxyproline. Pl. Physiol. 42: 271-4.

38. Crofton, H. D. 1966. Nematodes. Hutchison and Co. (Publishers) Ltd. London.

39. Curtis, R. W. 1969. Oxygen Requirement for Ethane Production in vitro by Phaseolus vulgaris. Pl. Physiol. 44: 1368-70.

40. Dropkin, V. H. 1963. Cellulose in Phytoparasitic Nematodes. Nematologica 9: 444-54.

41. Dropkin, V. H. and Nelson, P. E. 1960. The Histopathology of Root-Knot Nematode Infections in Soybeans. Phytopathol. 50: 442-7.

42. Dropkin, V. H., Helgeson, J. P. and Upper, C. D. 1969. The Hypersensitive Reaction of Tomatoes Resistant to the Root-Knot Nematode, Meloidogyne incognita. Nematologica 21: 95-103.

43. Durham, J. L., Morgan, P. W., Prescott, J. M. and Lyman, C. M. 1972. Enzymatic Synthesis of Ethylene in Germinating Peanut Seed (abst.) Pl. Physiol. Proc. 49: 5-21.

44. Eisinger, W. and Burg, S. P. 1972. Ethylene Induced Pea Internode Swelling. Pl. Physiol. 50: 510-17.

45. Elstner, E. F. and Konz, J. R. 1976. Effect of Point Freezing on Ethylene and Ethane Production by Sugar Beet Leaf Disks. Nature 263: 351-2.

46. Elstner, E. F. , Konz, J. R., Selman, B. R., and Stoffer, C. 1976. Ethylene Formation in Sugar Beet Leaves. Evidence for the Involvement of 3-Hydroxy-tyrosine and Phenoloxidase after Wounding. Pl. Physiol. 58: 163-8.

47. Endo, B. Y. 1971. Nematode Induced Syncytia (Giant Cells). Host-Parasite Relationships of Heteroderidae in Plant Parasitic Nematodes. (B. M. Zuckerman, W. F. Mai, R. A. Rhode eds.) Vol. 2, pp. 91-117.

48. Endo, B. Y. 1975. Pathogenesis of Nematode-Infected Plants. Ann. Rev. Phytopathol. 13: 213-38.

49. Endo, B. Y. and Veech, J. A. 1969. The Histochemical Localization of Oxidoreductive Enzymes of Soybeans Infested with the Root-Knot Nematode M. incognita acrita. Phytopathol. 59: 418-25.

50. Esashi, V. and Leopold, A. C. 1970. Dormancy Regulation in Subterranian Clover Seeds by Ethylene. Pl. Physiol. 44: 1470-2.

51. Farooq, T. 1973. The Anatomy of a Root Gall of Lycopersicon pimpinellifolium infected with Meloidogyne incognita. Nematologica 19: 118-9.

52. Fassuliotis, G., Deakin, J. R. and Hoffman, J. C. 1970. Root-Knot Nematode Resistance in Snap Beans: Breeding and Nature of Resistance. J. Amer. Hort. Sci. 95: 640-5.

53. Franklin, M. T. 1971. in Plant Parasitic Nematodes (B. M. Zuckermann, W. F. Mai and R. A. Rhode eds.) Vol. I, pp. 139-62. Academic Press, New York.

54. Frieser, L. F. and Frieser, M. 1967. Reagents for Organic Synthesis. John Wiley and Sons Inc., New York.

55. Gahagan, H. E., Holm, R. E. and Abeles, F. B. 1968. Effect of Ethylene on Peroxidase Activity. Physiol. Plant. 21: 1270-9.

56. Galliard, T., Hulme, A. C., Rhodes, M. J. C. and Wooltorton, L. S. C. 1968. Enzymic Conversion of Lineolenic Acid to Ethylene by Extracts of Apple Fruits. FEBS Letters 1: 283-6.

57. Galston, A. W. and Davies, P. J. 1970. Control Mechanisms in Plant Development. Prentice-Hall Inc., Englewood Cliffs, N. J.

58. Giebel, J. 1970. Phenolic Content in Roots of Some Solonaceae and Its Influence on IAA-Oxidase Activity as an Indicator of Resistance to Heterodera rostochiensis. Nematologica 16: 22-32.

59. _____ 1973. Phenylalanine and Tyrosine Ammonia-Lyase Activities in Potato Roots and Their Significance in Potato Sensitive to Heterodera Rostochiensis. Nematologica 19: 1-6.

60. _____ 1974. Biochemical Mechanisms of Plant Resistance to Nematodes: A Review. J. Nematol. 6: 175-84.

61. Giebel, J. and Stobiecka, M. 1974. Role of Amino Acids in Plant Tissue Response to Heterodera rostochiensis. 1. Protein-Proline and Hydroxyproline Content in Roots of Susceptible and Resistant Salanaceous Plants. Nematologica 20: 407-14.

62. Giebel, J., Kreg, J. and Wilski, A. 1971. Localization of Some Enzymes in Roots of Susceptible and Resistant Potatoes Infected with Heterodera rostochiensis. Nematologica 17: 28-33.

63. Giebel, J., Prigot, M. and Wilski, A. 1966. The Influence of Some Exogenic Enzymes on Root Tissues of Potatoes Susceptible and Resistant to the Golden Nematode (Heterodera rostochiensis). (Pr. Nauk. Inst. Ochr. Rasl. Warszawa 8: 204-11) in Giebel 1974.

64. Goodey, T. 1951. Laboratory Methods for Work with Plant and Soil Nematodes. Her Majesty's Stationery Office, London.

65. _____ 1963. Soil and Freshwater Nematodes. John Wiley and Sons Inc., New York.

66. Huang, C. S. 1966. Host-Parasite Relationship of the Root-Knot Nematode in Edible Ginger. Phytopathol. 56: 755-9.

67. Huang, C. S. and Maggenti, A. R. 1969a. Mitotic Aberrations and Nuclear Changes of Developing Giant Cells in Vicia faba caused by Root-Knot Nematode Meloidogyne javanica. Phytopathol. 59: 447-55.

68. Huang, C. S. and Maggenti, A. R. 1969b. Wall Modifications in Developing Giant Cells of Vicia faba and Cucumis sativus induced by Root-Knot Nematode Meloidogyne javanica. Phytopathol. 59: 931-7.

69. Hung, C. L. and Rhode, R. A. 1973. Phenol Accumulation Related to Resistance in Tomato to Infection by Root-Knot and Lesion Nematodes. J. Nematol. 5: 253-8.

70. Hussey, R. S. and Sasser, J. N. 1973. Peroxidase from Meloidogyne incognita. Physiol. Pl. Pathol. 3: 223-9.

71. Imaseki, H. 1970. Induction of Peroxidase Activity by Ethylene in Sweet Potato. Pl. Physiol. 46: 172-4.

72. Imaseki, H. and Burg, S. P. 1972. in Ethylene in Plant Growth (S. P. Burg). Proc. Nat. Acad. Sci. U.S.A. 70: 591-7.

73. Imaseki, H. and Pjon, C. J. 1970. The Effect of Ethylene on Auxin-Induced Growth of Excised Rice Coleoptile Segments. Plant and Cell Physiol. 11: 827-9.

74. Jatala, P. and Jensen, H. J. 1976. Histopathology of Beta vulgaris to Individual and Concomitant Infections of Meloidogyne hapla and Heterodera schachtii. J. Nematol. 8: 336-41.

75. Jatala, P. and Russell, C. C. 1972. Nature of Sweet Potato Resistance to Meloidogyne incognita and the Effects of Temperature on Parasitism. J. Nematol. 4: 1-7.

76. John, W. W. and Curtis, R. W. 1977. Isolation and Identification of the Precursor of Ethane in Phaseolus vulgaris L. Pl. Physiol. 59: 521-2.

77. Jones, M. G. K. and Northcote, D. H. 1972. Nematode-Induced Syncytia: A Multinucleate Transfer Cell. J. Cell. Sci. 10: 789-809.

78. Kang, B. G. and Burg, S. P. 1972. Relation of Phytochrome Enhanced Geotropic Sensitivity to Ethylene Production. Pl. Physiol. 50: 132-5.

79. Kang, B. G. and Ray, P. M. 1969. Ethylene and Carbon Dioxide as Mediators in the Response of Bean Hypocotyl Hook to Light and Auxins. Planta 87: 206-216.

80. Klinger, J. 1965. On the Orientation of Plant Nematodes and Some Other Soil Animals. Nematologica 11: 4-18.

81. Kochba, J. and Samish, R. M. 1971. Effect of Kinetics and 1-Naphthylacetic acid on Root-Knot Nematodes in Resistant and Susceptible Peach Root-Stocks. J. Am. Soc. Hort. Sci. 97: 115-9.

82. Kochba, J. and Samish, R. M. 1972. Level of Endogenous Cytokinins and Auxin in Roots of Nematode Resistant and Susceptible Peach Rootstocks. J. Am. Soc. Hort. Sci. 97: 115-119.

83. Krishnamoorthy, H. N. 1970. Promotion of Rooting in Mung Bean Hypocotyl Cuttings with Ethrel, an Ethylene Releasing Compound. Plant Cell Physiol. 11: 979-82.

84. Krusberg, L. R. 1963. Host Response to Nematode Infection. Ann. Rev. Phytopathol. 1: 219-40.

85. Krusberg, L. R. and Nielson, L. W. 1958. Pathogenesis of Root-Knot Nematodes to the Puerto Rico Variety of Sweet Potato. Phytopathol. 48: 30-39.

86. Ku, H. S., Yang, S. F. and Pratt, H. K. 1969. Ethylene Formation from α-Keto-γ-methylthiobutyrate by Tomato Fruit Extracts. Phytochemistry 8: 567-73.

87. Lee, D. L. 1965. The Physiology of Nematodes. W. H. Freeman and Co., San Francisco.

88. Lieberman, M. and Mapson, L. W. 1972. Fatty Acid Control of Ethane Production of Sub-Cellular Particles from Apples and its Possible Relationship to Ethylene Biosynthesis. Nature 195: 1016-17.

89. Lieberman, M., Kunishi, A. T., Mapson, L. W. and Wardale, D. A. 1966. Ethylene Production from Methionine. Biochem. J. 97: 449.

90. Littrell, R. H. 1966. Cellular Responses of Hibiscus esculentus to Meloidogyne incognita acrita. Phytopathol. 56: 540-4.

91. Loveys, B. R. and Bird, A. F. 1973. The Influence of Nematodes on Photosynthesis in Tomato Plants. Physiol. Pl. Pathol. 3: 525-529.

92. Lyon, C. J. 1970. Ethylene Inhibition of Auxin Transport by Gravity in Leaves. Pl. Physiol. 45: 644-6.

93. Matsuyama, N. and Misawi, T. 1963. Studies on the Hypertrophic Diseases Caused by Taphina Species. II. A Plant Growth Substance Excreted by the Pathogens. Tohoku J. Agr. Res. 14: 1-12.

94. Meheriuk, M. and Spencer, M. 1964a. Ethylene Production During Germination of Oat Seeds and Penicillium digitatum spores. Can. J. Bot. 42: 337-40.

95. _____ 1964b. Effects of Nitrogen and of Respiratory Inhibitors on Ethylene Production by a Sub-Cellular Fraction from Tomatoes. Nature 204: 337-8.

96. _____ 1967a. Studies on Ethylene Production by a Sub-Cellular Fraction From Ripening Tomatoes. I. Effects of Several Substrates, Cofactors and Cations. Phytochemistry 6: 535-44.

97. _____ 1967b. Studies on Ethylene Production by a Sub-Cellular Fraction From Ripening Tomatoes. II. Effects of Several Inhibitors. Phytochemistry 6: 545-58.

98. Mendenhall, W. 1975. Introduction to Probability and Statistics. 4th ed. Duxbury Press, Massachusetts.

99. Miles, P. W. 1968. Studies on the Salivary Physiology of Plant Bugs: Experimental Induction of Galls. J. Insect Physiol. 14: 97-106.

100. Miles, P. W. and Lloyd, J. 1967. Synthesis of a Plant Hormone by the Salivary Apparatus of Plant-Sucking Hemyptera. Nature 213: 801-2.

101. Moore, W. J. 1972. Physical Chemistry. 4th ed. Prentice-Hall Inc., Englewood Cliffs, N. J.

102. Mountain, W. B. 1960a. Mechanisms Involved in Plant Nematode Relationships in Nematology (J. N. Sasser and W. R. Jennings, eds.). U. of North Carolina Press, Chapel Hill, N. C. pp. 426-31.

103. Mountain, W. B. 1960b. Theoretical Considerations of Plant Nematode Relationships in Nematology. (J. N. Sasser and W. R. Jenkins, eds.) U. of North Carolina Press, Chapel Hill, N. C. pp. 419-21.

104. Mountain, W. B. and Patrick, Z. A. 1959. The Peach Replant Problem in Ontario. 7. The Pathogenicity of Pratylenchus penetrans. Can. J. Botany 37: 459-70.

105. Murdoch, J., Barnes, J. A. 1970. Statistical Tables for Science, Engineering Management and Business Science. 2nd ed. Macmillan Press Ltd., Toronto.

106. Naylor, J., Sander, G. and Skoog, F. 1954. Mitosis and Cell Enlargement Without Cell Division in Excised Tobacco Pith Tissue. Physiol. Plant. 7: 25.

107. Olsen, E. O., Rogers, B. and Rasmussen, G. K. 1970. Abscission of Citrus Fruit and Ethylene Production by Clementine Tangerine Fruit Grafted onto Seedlings infested with Stubborn Virus. Phytopathol. 60: 155-157.

108. Olthof, Th. H. A. and Potter, J. W. 1977. Effects of Population Densities of Meloidogyne hapla on Growth and Yield of Tomato. J. Nematol. 9: 296-300.

109. Orion, D. 1973. Studies on Plant Root-Knot Nematode Interrelationships. OEPP/EPPO Bulletin #9: 67-71.

110. Orion, D. and Bronner, R. 1973. The Localization of Starch Amylase and Invertase in Meloidogyne javanica Galls. Nematol. 19: 401-2.

111. Orion, D. and Hoestra, H. 1974. The Effect of Root-Knot Nematodes and Ethrel on Fusarium Wilt of Tomatoes. Neth. J. Pl. Pathol. 80: 28-36.

112. Orion, D. and Minz, G. 1968. The Influence of Systemic Plant Growth Substances on the Galls Induced by the Root-Knot Nematode Meloidogyne javanica. Phytopathol. 58: 384-5.

113. _____ 1969. The Effect of Ethrel (2-chloro-ethane Phosphoric Acid) on the Pathogenicity of the Root-Knot Nematode Meloidogyne javanica. Nematologica 15: 608-14.

114. Owens, R. G. and Rubenstein, J. N. 1966. Metabolic Changes Induced by Root-Knot Nematodes in Host Tissue. Contr. Boyce Thompson Inst. 23: 199-213.

115. Owens, R. G. and Specht, H. N. 1964. Root-Knot Histogenesis. Contr. Boyce Thompson Inst. 22: 471-87.

116. _____ 1966. Biochemical Alterations in Host Tissues by Root-Knot Nematodes. Contr. Boyce Thompson Inst. 23: 181-98.

117. Palmer, J. H. 1972. Roles of Ethylene and Indole-3-acetic Acid in Petiole Epinasty in Helianthus Annuus and The Modifying Influence of Gibberellic Acid. J. Exp. Bot. 23: 733-43.

118. Paulson, R. E. and Webster, J. M. 1970. Giant Cell Formation in Tomato Roots Caused by Meloidogyne incognita and Meloidogyne hapla (Nematode) Infection. A Light and Electron Microscope Study. Can. J. Bot. 48: 271-6.

119. _____ 1972. Ultrastructure of the Hypersensitive Reaction in Roots of Tomato, Lycopersicon esculent um L. to infection by the Root-Knot Nematode Meloidogyne incognita. Physiol. Pl. Pathol. 2: 227-34.

120. Peacock, F. E. 1960. Inhibition of Root-Knot Development on Tomato by Systemic Compounds. Nematologica 5: 219-27.

121. Pegg, G. F. and Cronshaw, D. K. 1976. Ethylene Production in Tomato Plants Infected with Verticillium albo-atrum. Physiol. Pl. Pathol. 8: 279-95.

122. Pitcher, R. S., Patrick, Z. A. and Mountain, W. B. 1960. Studies on the Host-Parasite Relations of Pratylenchus penetrans to Apple Seedlings. Nematologica 5: 309-14.

123. Reynolds, H. W., Carter, W. W. and O'Bannon, J. H. 1970. Symptomless Resistance of Alfalfa to Meloidogyne Incognita acrita. J. Nematol. 2: 131-4.

124. Rickels, J. W. 1973. Nutrient Solutions for Hydroponics. Ontario Ministry of Agriculture and Food. Factsheet 200-532.

125. Ridge, I. and Osborne, D. J. 1970. Regulation of Peroxidase Activity by Ethylene in Pisum sativum: Requirements for Protein and RNA Synthesis. J. Exp. Bot. 21: 720-34.

126. Riedel, R. M. and Mai, W. F. 1971. Pectinases in Aqueous Extracts of Ditylenchus dipsaci. J. Nematol. 3: 28-38.

127. Riggs, R. D., Kimm, K. S. and Gipson, I. 1973. Ultrastructural Changes in Peking Soybeans Infested with Heterodera glycines. Phytopathol. 63: 76-84.

128. Riggs, R. D. and Winstead, N. N. 1959. Studies on Resistance in Tomato to Root-Knot Nematodes and on the Occurence of Pathogenic Biotypes. Phytopathol. 19: 716-24.

129. Riov, J., Monselise, S. P. and Kahan, R. S. 1969. Ethylene Controlled Induction of Phenylalanine Ammonia-Lyase in Citrus Fruit Peel. Pl. Physiol. 44: 631-5.

130. Rubenstein, J. H. and Owens, R. G. 1964. Thymidine and Uridine Incorporation in Relation to the Ontogeny of Root-Knot Syncytia. Contr. Boyce Thompson Inst. 22: 491-502.

131. Sandstedt, R. and Schuster, M. L. 1966. Excised Tobacco Pith Bioassays for Root-Knot Nematode Produced Plant Growth Substances. Physiol. Plant. 19: 99-104.

132. Sasser, J. N. and Jenkins, W. R. 1960. Nematology. U. of North Carolina Press, Chapel Hill, N. C.

133. Sawhney, R. and Webster, J. M. 1975. The Role of Plant Growth Hormones in Determining the Resistance of Tomato Plants to the Root-Knot Nematode, Meloidogyne incognita. Nematologica 21: 95-103.

134. Seinhorst, J. W. 1961. Plant-Nematode Interrelationships. Ann. Rev. Microbiol. 15: 177-96.

135. Sequeira, L. 1973. Hormone Metabolism in Diseased Plants. Ann. Rev. Pl. Physiol. 24: 353-80.

136. Setty, K. G. J. and Wheeler, A. W. 1968. Growth Substances in Roots of Tomato (Lycopersicon esculentum) Infected with Root-Knot Nematodes (Meloidogyne spp.) Ann. of App. Biol. 61: 495-501.

137. Shain, L. and Hillis, W. E. 1972. Ethylene Production in Pinus radiata in Response to Sirex amylostereum Attack. Phytopathol. 62: 1407-9.

138. Seigel, S. M. 1953. Biosynthesis of Lignins. Physiol. Plant. 6: 134-9.

139. Singh, B. and Choudhury, B. 1974. The Chemical Characteristics of Tomato Cultivars Resistant to Root-Knot Nematodes (Meloidogyne spp.) Nematologica 19: 443-8.

140. Stahmann, M. A., Clare, B. G. and Woodbury, W. 1966. Increased Disease Resistance and Enzyme Activity Induced by Ethylene and Ethylene Production by Black Rot Infected Sweet Potato Tissue. Pl. Physiol. 41: 1505-12.

141. Talboys, P. W. 1972. Resistance to Vascular Wilt Fungi. Proc. R. Soc. Lond. B. 181: 319-332.

142. Thorne, G. 1961. Principles of Nematology. McGraw-Hill Book Co., Inc., Toronto.

143. Tolbert, N. E. 1961. Standard Relationships Among Chemical Which Act Like Anti-Gibberellins. Adv. in Chem. Ser. 28: 145-51.

144. Toole, V. K., Bailey, W. K. and Toole, E. H. 1964. Factors Influencing Dormancy of Peanut Seeds. Pl. Physiol. 39: 822-32.

145. Tomaszewicki, M. and Thimann, K. F. 1966. Interaction of Phenolic Acids, Metallic Ions and Chelating Agents on Auxin-Induced Growth. Pl. Physiol. 14: 1443-54.

146. Townshend, J. L. and Baenziger, H. 1976. Evidence of Resistance to Root-Knot and Root-Lesion Nematodes in Alfalfa Clones. Can. J. Plant Sci. 56: 977-9.

147. Triantaphyllou, A. C. 1973. Environmental Sex Differentiation of Nematodes in Relation to Pest Management. Ann. Rev. Phytopathol. 11: 441-62.

148. Uhlenbroek, J. H. and Bijloo, J. D. 1959. Isolation and Structure of a Nematocidal Principle Occurring in Tagetes Roots. from Giebel, J. 1974.

149. Veech, J. A. and Endo, B. Y. 1969a. The Histochemical Localization of Several Enzymes of Soybeans Infected with the Root-Knot Nematode, Meloidogyne incognita acrita. J. Nematol. 1: 265-76.

150. _____ 1969b. Alterations in Enzyme Localization in Soybeans Infected with Meloidogyne incognita acrita. J. Nematol. 1: 307-8.

151. Viglierchio, D. R. 1971. Nematodes and Other Pathogens in Auxin Related Plant Growth Disorders. Bot. Review. 37: 1-21.

152. Viglierchio, D. R. and Yu, P. K. 1965. Plant Parasitic Nematodes: A New Mechanism for Injury of Hosts. Science. 147: 1301-3.

153. _____ 1968. Plant Growth Substances in Plant Parasitic Nematodes. Host Influence on Auxin Content. Exp. Parasitol. 23: 88-95.

154. Wang, E. C. H. and Hughes, I. W. 1976. A Stem Gall of Cactus Induced by Meloidogyne incognita. Pl. Disease Reporter 60: 906-7.

155. Weast, R. C. ed. 1972. Handbook of Chemistry and Physics, 53rd ed. The Chemical Rubber Co., Cleveland.

156. Webster, J. M. 1969. The Influence of Plant Growth Substances and Their Inhibitors on the Host-Parasite Relationships of Aphelenchoides ritzemabosi in Culture. Nematologica 13: 256-62.

157. Webster, J. M. 1975. Aspects of the Host-Parasite Relationship of Plant Parasitic Nematodes. Advances in Parasitol. 13: 225-50.

158. Webster, J. M. and Paulson, R. E. 1972. An Interpreation of the Ultrastructure of Response of Tomato Roots Susceptible and Resistant to Meloidogyne incognita. OEPP/EPPO Bull. No. 6: 33-39.

159. Webster, P. L. and Davidson, D. 1967. Studies on IAA Effects on the S Plase of the Mitotic Cycle. Am. J. Bot. 54: 633.

160. Weise, M. V. and DeVay, J. E. 1970. Growth Regulation Changes in Cotton Associated with Defoliation caused by Verticillium albo-atrum. Pl. Physiol. 45: 304-9.

161. Wickson, M. and Thimann, K. V. 1958. The Antagonism of Auxin and Kinetin in Apical Dominance. Physiol. Plant. 11: 62-74.

162. Wilski, A. and Giebel, J. 1966. β-Glucosidase in Heterodera rostochiensis and its Significance in Resistance of Potato to this Nematode. Nematologica 12: 219-24.

163. Wilski, A. and Giebel, J. 1971. The Destruction of IAA in Roots of Potatoes Susceptible and Resistant to Heterodera rostochiensis. from Giebel 1974.

164. _____ 1972. Phenolics in Potato Roots and Their Influence on Susceptible-Resistant Reactions to Heterodera rostochiensis. from Giebel 1974.

165. Winstead, N. N. and Riggs, R. D. 1963. Stability of Pathogenicity of B Biotypes of the Root-Knot Nematode Meloidogyne incognita on Tomato. Pl. Disease Reporter 47: 870-1.

166. Yu, P. K. and Viglierchio, D. R. 1964. Plant Growth Substances and Parasitic Nematodes. I. Root-Knot Nematodes and Tomato. Exp. Parasitol. 15: 242-8.

8. Appendices

Appendix I. Experimental Raw Data

Table 1. Experiment 1 Susceptible Tomato Plants

Experiment Run Number	Time (days)	Stem Height (cm)	Top Weight (g)	Root Weight (g)	Volume of Gas (ml)	C_2H_4 Peak Size (counts)	C_2H_4 (ng/g root)	C_2H_6 Peak Size (counts)	C_2H_6 (ng/g root)
1-1C	2	6.9[1]	2.9[1]	3.0	1.93	2.5	0.075	19	0.80
1-2C		6.0[2]	2.1[2]	2.8	1.35	1.1	0.030	28	1.22
1-1I		7.4[1]	2.9[1]	3.4	2.80	1.7	0.065	35	2.28
1-2I		6.0	2.0	2.0	1.28	3.0	0.13	44	3.47
2-1C	4	7.5	3.3	4.5	1.70	1.7	0.045	105	5.39
2-2C		7.3	2.9	3.1	0.85	3.6	0.069	90	3.34
2-1I		7.3	3.0	4.2	1.65	2.4	0.066	46	2.26
2-2I		7.4	2.9	4.1	1.70	1.8	0.056	60	3.19
3-1C	7	7.6	3.6	3.8	1.60	2.5	0.073	80	4.52
3-2C		7.9	4.3	6.9	1.20	3.3	0.040	78	1.78
3-1I		8.3	4.0	4.2	1.20	2.0	0.040	70	2.65
3-2I		8.7	4.5	5.2	1.40	3.5	0.065	68	2.42
4-1C	9	8.9	4.8	5.5	1.90	7.1	0.17	76	3.44
4-2C		8.9	4.6	7.0	1.34	10.0	0.13	121	3.18
4-1I		8.4	4.0	5.2	1.36	6.8	0.12	76	2.64
4-2I		7.8	3.5	4.9	1.00	5.5	0.078	75	2.01
5-1C	11	9.2	4.6	3.8	1.20	1.4	0.031	33	1.23
5-2C		9.1	5.1	4.8	1.25	9.5	0.17	104	3.64
5-1I		8.0	3.7	3.9	0.92	6.8	0.12	39	1.11
5-2I		9.0	6.0	5.9	1.50	5.5	0.097	30	0.87
6-1C	13	10.3	5.8	5.9	1.73	6.8	0.19	55	2.06
6-2C		11.3	5.0	5.0	1.50	3.7	0.077	53	2.02
6-1I		10.1	4.3	5.5	1.33	7.2	0.12	77	2.47
6-2I		11.5	5.8	5.9	1.35	5.8	0.092	52	1.50
7-1C	15	10.7	5.8	4.3	1.91	6.9	0.21	80	4.74
7-2C		11.9	5.8	4.8	1.66	7.5	0.18	84	3.91
7-1I		10.8	5.8	5.0	1.70	4.4	0.10	81	3.71
7-2I		10.8	5.7	4.7	1.27	5.9	0.11	65	2.29
8-1C	17	11.9	8.2	8.6	0.61	20.8	0.10	60	0.55
8-2C		12.0	10.0	6.8	0.60	68.5	0.42	122	1.47
8-1I		11.9	6.5	4.3	0.70	34.7	0.39	67	1.43
8-2I		12.7	9.8	7.3	0.47	29.8	0.13	73	0.61
9-1C	18	12.4	7.9	5.4	0.40	53.8	0.28	290	3.04
9-2C		13.0	8.0	4.9	0.41	17.9	0.10	189	2.19
9-1I		12.6	7.4	4.8	0.39	25.7	0.15	186	2.08
9-2I		12.6	7.5	5.1	0.31	43.3	0.18	250	2.14
10-1C	20	15.1	11.8	9.1	0.49	16.1	0.060	167	1.24
10-2C		13.1	8.9	7.1	0.36	12.1	0.043	116	0.80
10-1I		12.6	10.2	6.6	0.40	14.3	0.060	151	1.27
10-2I		13.4	10.2	8.7	0.39	--	--	79	0.47
11-1C	22	14.1	8.4	6.7	0.50	17.5	0.091	176	1.81
11-2C		14.2	9.5	8.6	0.37	29.3	0.082	371	2.25
11-1I		11.5	8.3	10.0	0.47	22.2	0.072	389	2.59
11-2I		13.4	10.2	--	0.44	--	--	--	--
12-1C	25	14.0	10.0	9.9	0.72	11.9	0.060	273	2.79
12-2C		13.2	11.4	9.9	0.46	7.9	0.026	278	1.81
12-1I		13.3	11.0	10.9	0.34	10.7	0.23	403	1.78
12-2I		14.5	10.6	10.8	0.44	9.8	0.028	218	1.24
13-1C	27	16.0	13.6	11.3	0.51	7.6	0.024	106	0.65
13-2C		15.3	10.9	10.1	0.44	5.0	0.015	175	1.06
13-1I		14.5	11.3	10.1	0.45	4.4	0.014	105	0.62
13-2I		14.5	11.2	8.5	0.39	6.6	0.021	206	1.32
14-1C	29	17.9	13.2	11.5	0.64	6.8	0.026	320	2.50
14-2C		15.9	13.7	12.0	0.45	4.2	0.011	251	1.32
14-1I		15.0	14.3	7.5	0.55	7.3	0.037	--	--
14-2I		14.2	11.2	7.9	0.38	2.4	0.008	223	1.50
15-1C	36	16.5	13.5	14.4	0.74	8.3	0.032	48	0.31
15-2C		16.4	14.2	15.1	1.15	8.0	0.042	30	0.26
15-1I		18.6	17.9	12.3	1.26	13.3	0.095	60	0.80
15-2I		18.5	16.8	13.3	--	--	--	--	--
16-1C	43	24.0	19.7	12.6	1.00	27.0	0.15	81	0.85
16-2C		25.4	22.1	13.3	--	--	--	--	--
16-1I		22.0	24.2	14.3	--	--	--	--	--
16-2I		21.5	20.9	13.1	0.99	20.9	0.11	162	1.69

[1] Indicates the mean value for 6 plants
[2] Indicates the mean value for 5 plants
All other stem heights and top weights are the mean values for 4 plants. Root weight is the total weight of all roots used in the experimental run. Total gas is that extracted from all roots of the run.
-- Data not available due to technical problems with the electronic recorder or gas extraction apparatus.
Experiment conducted January/February, 1977.

Table 2. Experiment 2. Susceptible Tomato Plants

Experiment Run Number	Time (days)	Stem Height (cm)	Top Weight (g)	Root Weight (g)	Volume of Gas (ml)	C_2H_4 Peak Size (counts)	C_2H_4 (ng/g root)	C_2H_6 Peak Size (counts)	C_2H_6 (ng/g root)
1-1C	0.08	24.8	17.1	11.9	0.56	11.0	0.036	84	0.53
1-2C		28.5	20.3	11.6	0.93	16.7	0.093	88	0.95
1-1I		26.8	17.5	11.5	0.99	17.9	0.11	94	1.09
1-2I		28.3	22.0	12.9	0.76	6.1	0.025	34	0.24
2-1C	0.80	30.5	20.7	9.6	1.11	21.3	0.17	149	2.36
2-2C		28.7	20.1	10.0	0.49	8.9	0.030	152	1.02
2-1I		32.8	23.3	10.9	0.35	3.3	0.0074	90	0.39
2-2I		26.6	18.7	10.7	0.84	21.3	0.090	157	1.70
3-1C	1.33	33.5	20.1	10.2	0.79	13.3	0.072	176	1.89
3-2C		30.0	17.4	9.5	1.38	25.3	0.26	172	3.46
3-1I		31.2	25.4	14.5	1.00	23.0	0.11	188	1.80
3-2I		27.1	17.0	9.8	0.74	15.0	0.079	211	2.22
4-1C	2	33.4	20.6	10.2	0.72	14.3	0.070	187	1.83
4-2C		31.0	18.7	10.1	1.36	3.9	0.037	160	2.98
4-1I		25.9	18.4	10.4	0.64	20.8	0.089	257	2.22
4-2I		30.8	22.1	14.0	0.47	20.0	0.047	111	0.50
5-1C	3	32.0	20.5	11.3	1.07	25.5	0.17	177	2.32
5-2C		29.5	17.7	9.9	0.84	12.5	0.074	130	1.51
5-1I		30.6	23.5	15.6	0.75	8.6	0.029	184	1.23
5-2I		28.9	18.2	8.6	0.96	25.0	0.19	180	2.78
6-1C	4	31.6	16.5	9.5	0.87	9.4	0.060	165	2.09
6-2C		33.6	27.4	15.1	0.99	20.5	0.093	197	1.80
6-1I		33.0	21.0	11.7	1.20	21.5	0.15	202	2.88
6-2I		32.7	22.5	12.2	1.12	7.8	0.050	154	1.95
7-1C	8	29.8	20.9	12.9	0.91	57.7	0.28	65	0.60
7-2C		31.9	17.9	10.0	1.02	25.6	0.18	128	1.79
7-1I		32.3	26.1	15.5	1.17	23.0	0.12	111	1.13
7-2I		32.3	24.8	11.1	0.48	40.0	0.12	87	0.50
8-1C	11	35.4	18.2	9.5	0.96	22.4	0.16	75	1.00
8-2C		33.6	19.1	9.6	0.65	5.8	0.027	73	0.65
8-1I		35.4	22.7	12.8	0.64	4.2	0.015	21	0.11
8-2I		30.0	20.2	10.3	0.67	42.9	0.19	151	1.35
9-1C	12	29.8	24.9	14.6	0.56	5.5	0.015	54	0.264
9-2C		33.2	17.3	10.2	1.39	26.5	0.25	90	1.64
9-1I		33.9	27.5	13.0	1.32	16.0	0.11	69	0.92
9-2I		33.1	20.0	11.3	0.52	10.9	0.035	98	0.61
10-1C	14	31.8	19.2	11.3	1.62	25.5	0.25	139	2.74
10-2C		37.5	21.4	9.9	1.46	32.8	0.34	134	2.70
10-1I		35.8	26.6	13.2	1.48	60.8	0.47	206	3.22
10-2I		37.0	33.5	15.4	2.07	37.5	0.35	156	2.89
11-1C	15	34.7	18.3	11.1	1.57	84.5	0.83	201	3.95
11-2C		34.5	21.6	14.6	2.30	62.5	0.68	204	4.47
11-1I		39.0	26.9	13.2	1.54	71.5	0.58	192	3.10
11-2I		36.9	26.2	13.3	2.02	76.5	0.81	214	4.53
12-1C	16	30.8	--	10.9	1.60	70.8	0.72	160	3.24
12-2C		34.0	20.8	10.7	1.74	59.0	0.67	132	2.94
12-1I		38.6	--	14.4	1.58	63.3	0.48	144	2.17
12-2I		40.3	26.4	12.2	1.61	53.5	0.49	142	2.57
13-1C	19	44.0	28.9	15.2	1.71	70.0	0.55	119	1.82
13-2C		37.9	26.4	12.2	1.81	74.5	0.77	163	3.34
13-1I		41.3	26.2	11.1	1.04	87.0	0.57	171	2.21
13-2I		41.1	29.2	14.2	1.39	88.0	0.44	172	1.72
14-1C	22	47.5	35.0	12.1	0.98	60.0	0.34	106	1.16
14-2C		39.9	25.0	12.6	1.16	76.0	0.49	237	3.05
14-1I		44.3	39.9	15.9	1.13	102.0	0.50	182	1.79
14-2I		47.3	33.2	12.4	1.06	79.0	0.47	142	1.67
15-1C	25	44.6	40.4	14.4	1.34	62.0	0.40	177	2.28
15-2C		50.1	50.7	18.4	1.59	58.0	0.35	101	1.18
15-1I		45.4	35.9	16.2	1.68	74.0	0.53	134	1.90
15-2I		44.2	37.6	20.0	1.75	43.0	0.26	95	1.12
16-1C	26	50.4	36.5	14.7	1.44	43.0	0.29	100	1.32
16-2C		49.8	43.7	15.8	1.57	54.0	0.37	133	0.86
16-1I		48.4	39.8	16.0	1.54	56.0	0.37	115	1.50
16-2I		40.0	33.9	16.9	1.89	46.0	0.36	125	1.91
17-1C	27	52.0	45.2	6.9	0.87	102.0	0.89	134	2.31
17-2C		54.5	47.3	6.7	0.59	94.0	0.58	118	1.42
17-1I		51.0	44.8	7.9	0.67	124.0	0.73	222	2.62
17-2I		46.0	40.8	9.4	1.18	63.0	0.55	104	1.76

continued, p. 2

Table 2, p. 2

Experiment Run Number	Time (days)	Stem Height (cm)	Top Weight (g)	Root Weight (g)	Volume of Gas (ml)	C_2H_4 Peak Size (counts)	C_2H_4 (ng/g root)	C_2H_6 Peak Size (counts)	C_2H_6 (ng/g root)
18-1C	28	53.3	46.7	8.4	0.75	112.0	0.70	210	2.61
18-2C		50.7	47.0	8.7	0.68	63.0	0.34	154	1.66
18-1I		53.0	49.9	9.0	0.69	88.0	0.47	134	1.40
18-2I		45.5	44.4	9.8	0.87	64.0	0.40	181	2.23
19-1C	46	67.2	71.9	12.5	0.90	86.0	0.43	148	1.46
19-2C		73.3	61.6	8.0	0.82	116.0	0.83	178	2.52
19-1I		54.4	58.4	12.4	1.10	122.0	0.75	210	2.59
19-2I		56.3	69.4	16.1	1.19	74.0	0.38	138	1.40

Stem Heights and Top Weights are the mean values for 2 plants per experimental run up to and including run 16. Run 17 and on are the values of a single plant used in each experimental run.

Root Weight is the total weight of all roots used in the experimental run.
Total gas is that extracted from all roots of the experimental run
--Record of this data lost.
Experiments conducted May/June 1977.

Table 3. Experiment 3. Susceptible Tomato Plants

Experiment Run Number	Time (days)	Stem Height (cm)	Top Weight (g)	Root Weight (g)	Volume of Gas (ml)	C_2H_4 Peak Size (counts)	C_2H_4 (ng/g root)	C_2H_6 Peak Size (counts)	C_2H_6 (ng/g root)
1-1C	0.25	62.0	48.1	5.2	0.62	89.0	0.74	66	1.03
1-2C		41.0	37.7	5.5	0.32	56.5	0.23	47	0.34
1-1I		63.5	44.0	3.5	0.27	96.0	0.52	64	0.64
1-2I		51.0	41.0	4.4	0.67	70.0	0.74	56	1.09
2-1C	1	55.0	33.2	3.1	0.41	87.5	0.86	210	3.86
2-2C		45.0	37.7	4.2	0.29	66.0	0.29	76	0.69
2-1I		38.0	36.9	5.0	0.33	53.5	0.24	65	0.56
2-2I		60.0	39.5	3.5	0.47	55.5	0.52	77	1.37
3-1C	2	70.0	43.2	3.8	0.36	51.5	0.340	83	1.04
3-2C		36.0	34.1	5.0	0.23	50.0	0.16	190	1.21
3-1I		53.0	40.8	5.4	0.32	38.5	0.16	45	0.33
3-2I		53.5	40.6	4.1	0.49	62.5	0.52	95	1.53
4-1C	4	61.5	41.5	5.8	0.60	14.0	0.10	21	0.22
4-2C		53.0	40.9	4.8	0.27	12.0	0.047	62	0.45
4-1I		51.0	57.8	4.7	0.24	20.0	0.071	40	0.25
4-2I		66.5	62.2	7.4	0.87	12.0	0.098	21	0.25
5-1C	6	54.0	33.9	3.9	0.56	7.5	0.075	48	0.86
5-2C		61.0	36.0	2.9	0.21	17.5	0.088	50	0.46
5-1I		42.0	38.6	6.3	0.40	11.5	0.050	60	0.49
5-2I		59.5	51.5	5.9	0.74	7.0	0.061	29	0.41
6-1C	9	91.0	62.6	6.8	0.67	31.0	0.21	51	0.64
6-2C		70.0	42.6	5.2	0.96	24.0	0.31	103	2.57
6-1I		62.0	46.2	7.4	0.44	27.5	0.11	41	0.30
6-2I		63.0	55.1	9.4	0.40	17.0	0.051	43	0.23
7-1C	11	60.0	47.6	6.7	0.77	22.0	0.18	69	1.04
7-2C		75.0	46.8	5.6	0.67	22.0	0.18	26	0.34
7-1I		62.5	51.4	6.0	0.56	20.0	0.13	68	0.83
7-2I		69.0	35.8	3.6	0.60	14.0	0.16	38	0.77
8-1C	13	49.0	35.2	4.0	0.50	12.0	0.11	43.5	0.67
8-2C		68.0	55.8	7.3	0.51	23.0	0.11	66	0.60
8-1I		75.0	49.5	6.6	0.70	25.0	0.19	50	0.83
8-2I		52.5	41.5	7.4	0.71	22.0	0.15	76.5	0.97
9-1C	15	63.0	38.1	5.6	0.89	20.0	0.22	83	1.89
9-2C		72.0	52.9	7.6	0.52	42.0	0.20	126	0.45
9-1I		59.0	45.2	8.7	0.76	29.0	0.17	80	0.93
9-2I		72.0	67.0	9.1	1.07	21.5	0.21	79	1.23
10-1C	19	75.5	47.7	4.3	0.54	14.0	0.12	154	2.67
10-2C		70.0	55.3	5.4	0.35	23.5	0.11	90	0.73
10-1I		56.0	49.0	12.0	1.01	19.0	0.11	82.5	0.93
10-2I		70.0	48.3	11.7	1.18	15.0	0.11	60.5	0.79
11-1C	21	52.5	50.3	7.9	0.74	15.0	0.097	86.5	1.09
11-2C		100.0	53.0	6.5	0.56	25.5	0.15	72.5	0.82
11-1I		68.5	64.1	10.0	0.82	13.5	0.076	25.5	0.27
11-2I		61.0	48.6	13.3	1.02	7.5	0.039	41.5	0.39
12-1C	23	84.0	45.2	6.6	0.81	8.5	0.073	45.5	0.70
12-2C		70.0	62.3	7.7	0.40	17.0	0.062	60	0.40
12-1I		60.5	55.8	14.4	1.17	12.0	0.068	30	0.28
12-2I		67.5	54.1	13.8	1.20	7.0	0.042	50	0.55
13-1C	25	71.5	48.2	5.0	0.54	12.5	0.094	90	1.30
13-2C		62.0	38.1	5.8	0.38	18.5	0.085	79	0.68
13-1I		56.0	46.2	9.8	0.57	14.0	0.057	33	0.27
13-2I		69.0	44.3	15.5	1.64	6.5	0.048	48	0.64
14-1C	27	95.5	62.4	6.0	0.72	13.5	0.11	103.5	1.67
14-2C		85.0	49.4	4.6	0.27	20.0	0.082	107.5	0.85
14-1I		45.0	42.8	15.3	1.18	12.0	0.064	50	0.49
14-2I		76.0	71.3	24.3	2.20	6.0	0.038	34	0.36
15-1C	30	67.0	54.7	8.2	0.78	8.0	0.052	74.5	0.93
15-2C		54.0	30.0	3.5	0.21	20.0	0.083	120	1.01
15-1I		64.5	61.1	21.6	0.56	10.5	0.019	54.5	0.37
15-2I		81.5	63.7	12.2	0.74	6.5	0.027	70	1.04

Stem heights and gas peak sizes were measured to the nearest half unit.

All values measured per run were obtained from a single plant used.

Experiment conducted July/August 1977.

Table 4. Experiment 4. Resistant Tomato Plants

Experiment Run Number	Time (days)	Stem Height (cm)	Top Weight (g)	Root Weight (g)	Volume of Gas (ml)	C_2H_4 Peak Size (counts)	C_2H_4 (ng/g root)	C_2H_6 Peak Size (counts)	C_2H_6 (ng/g root)
1-1C	0.33	52.0	44.8	6.4	0.69	76.0	0.55	52.0	0.71
1-2C		55.5	54.6	6.3	0.44	40.0	0.19	28.0	0.22
1-1I		55.5	54.1	7.6	0.58	42.0	0.22	37.0	0.34
1-2I		62.0	61.7	7.3	0.91	43.0	0.37	34.0	0.50
2-1C	1	57.0	42.6	6.0	1.07	40.0	0.50	38.0	0.82
2-2C		55.5	80.2	12.1	0.91	44.0	0.23	45.0	0.42
2-1I		41.0	39.3	6.2	0.43	41.5	0.20	38.0	0.32
2-2I		55.0	38.7	3.6	0.58	52.0	0.58	62.0	1.29
3-1C	2	57.5	51.4	6.5	0.67	54.0	0.39	72.0	0.98
3-2C		42.0	46.0	6.5	0.40	50.0	0.21	77.0	0.63
3-1I		59.5	66.0	8.2	0.57	54.0	0.26	78.0	0.72
3-2I		63.5	62.4	7.0	0.79	53.0	0.42	74.0	1.10
4-1C	4	53.0	48.5	5.4	0.68	10.0	0.088	29.0	0.42
4-2C		43.0	34.9	5.7	0.26	8.0	0.025	28.0	0.14
4-1I		57.5	26.9	3.2	0.25	10.0	0.054	38.0	0.36
4-2I		57.5	68.7	9.0	0.77	8.0	0.048	22.0	0.20
5-1C	6	60.0	85.4	14.6	1.50	7.0	0.048	22.0	0.24
5-2C		59.5	52.8	6.7	0.81	13.5	0.11	47.0	0.71
5-1I		58.0	56.6	5.0	0.38	13.0	0.058	30.0	0.22
5-2I		61.0	64.6	6.0	1.02	10.5	0.12	38.0	0.73
6-1C	9	67.0	59.0	7.0	0.77	23.0	0.17	49.0	0.68
6-2C		63.0	86.1	16.0	1.03	16.0	0.072	34.0	0.26
6-1I		62.0	58.6	6.0	0.45	27.5	0.14	55.0	0.53
6-2I		66.0	73.7	10.5	1.08	18.0	0.13	45.0	0.58
7-1C	11	50.0	48.0	6.2	0.76	26.0	0.22	85.0	1.39
7-2C		60.0	79.8	10.8	0.81	20.0	0.10	41.0	0.38
7-1I		57.0	60.3	8.6	0.71	22.5	0.13	50.0	0.52
7-2I		68.5	42.5	5.2	0.54	17.5	0.13	55.0	0.73
8-1C	13	59.0	53.4	10.5	1.05	16.5	0.11	61.5	0.79
8-2C		59.0	48.8	7.8	0.32	26.5	0.076	71.5	0.39
8-1I		39.0	62.5	6.6	0.60	31.0	0.20	82.0	0.99
8-2I		66.0	57.2	9.2	1.01	18.0	0.14	53.5	0.75
9-1C	15	59.0	50.5	7.3	0.93	14.0	0.12	72.5	1.21
9-2C		54.0	45.2	5.0	0.42	39.0	0.23	107.5	1.22
9-1I		39.0	28.6	3.4	0.17	22.0	0.076	81.5	0.54
9-2I		66.0	66.1	8.0	1.14	16.5	0.17	78.0	1.47
10-1C	19	57.0	43.7	5.0	0.64	16.5	0.15	80.0	1.36
10-2C		48.5	44.3	5.6	0.43	15.0	0.080	66.5	0.67
10-1I		74.0	85.5	9.7	0.82	15.5	0.092	70.0	0.78
10-2I		71.0	56.1	6.9	0.69	14.0	0.097	76.0	1.00
11-1C	21	49.5	67.8	11.6	1.01	12.5	0.076	54.0	0.60
11-2C		71.5	47.3	4.3	0.33	17.5	0.094	62.5	0.62
11-1I		56.5	52.4	5.7	0.55	21.0	0.14	70.0	0.89
11-2I		69.0	58.6	7.0	0.88	13.0	0.11	56.5	0.88
12-1C	23	56.5	54.3	7.3	0.72	8.5	0.059	68.5	0.89
12-2C		70.5	69.8	10.0	0.72	22.5	0.11	52.5	0.48
12-1I		73.0	55.9	12.4	0.42	15.0	0.068	27.5	0.20
12-2I		64.0	76.5	6.4	1.15	9.0	0.057	43.0	0.49
13-1C	25	60.0	59.0	7.5	0.71	6.5	0.044	78.5	0.99
13-2C		57.0	77.4	13.4	0.82	12.0	0.051	52.0	0.40
13-1I		55.0	66.1	7.8	0.63	21.0	0.12	62.5	0.65
13-2I		84.0	92.1	14.7	1.58	7.5	0.056	68.0	0.96
14-1C	27	62.5	41.1	5.3	0.60	10.0	0.079	110.0	1.69
14-2C		51.0	53.6	6.4	0.48	15.0	0.078	52.5	0.50
14-1I		78.0	69.1	7.4	0.60	17.5	0.097	68.0	0.72
14-2I		77.5	88.4	14.9	1.35	6.0	0.038	58.0	0.68

Stem heights and gas peak sizes were measured to the nearest half unit.

All values measured per run are obtained from a single plant used.

Experiment conducted July/August 1977.

Table 5. Experiment 5. Susceptible Tomato Plants

Experiment Run Number	Time (days)	Stem Height (cm)	Top Weight (g)	Root Weight (g)	Volume Of Gas (ml)	C_2H_4 Peak Size (counts)	C_2H_4 (ng/g root)	C_2H_6 Peak Size (counts)	C_2H_6 (ng/g root)
1-1C	0.25	63.5	43.2	5.4	1.03	24	0.34	17	0.57
1-2C		74.5	46.8	4.9	0.28	33	0.16	33	0.36
1-1I		57.0	41.9	5.5	0.31	30	0.14	33	0.36
1-2I		69.0	53.7	7.1	1.36	23	0.32	19	0.66
2-1C	1.25	52.0	26.5	3.5	0.78	28	0.49	18	0.72
2-2C		59.0	47.5	6.5	0.57	34	0.25	60	1.06
2-1I		67.5	52.8	6.5	0.53	40	0.28	70	1.16
2-2I		45.0	57.4	11.0	1.57	24	0.25	25	0.67
3-1C	2	71.0	54.7	7.4	1.06	49	0.63	26	0.70
3-2C		48.0	24.5	3.9	0.26	35	0.20	80	1.09
3-1I		63.0	39.2	6.2	0.36	43	0.22	60	0.70
3-2I		56.0	59.7	10.7	1.32	49	0.54	42	1.02
4-1C	4	50.0	49.5	6.4	0.83	26	0.26	34	0.85
4-2C		52.5	33.6	7.3	0.30	45	0.16	80	0.67
4-1I		70.5	38.9	8.9	0.40	37	0.14	66	0.60
4-2I		54.0	38.8	4.2	1.04	37	0.78	48	2.36
5-1C	7	54.0	39.8	5.2	0.95	18	0.21	--	--
5-2C		68.5	61.0	9.4	0.58	54	0.30	126	1.60
5-1I		72.0	48.3	6.7	0.37	73	0.39	197	2.26
5-2I		60.0	45.1	8.8	0.92	47	0.44	91	1.94
6-1C	9	72.0	59.0	8.5	0.38	45	0.47	71	1.71
6-2C		66.0	36.8	6.2	0.50	76	0.59	142	2.36
6-1I		67.5	52.2	7.3	0.78	116	1.23	144	3.18
6-2I		76.0	45.7	5.4	0.64	57	0.62	125	3.06
7-1C	11	65.0	43.0	5.8	1.23	46	0.87	170	7.46
7-2C		71.5	38.4	5.7	0.50	62	0.51	187	3.40
7-1I		78.0	49.0	6.0	0.58	40	0.33	160	3.20
7-2I		65.0	41.1	6.1	0.77	51	0.58	163	4.27
8-1C	13	60.0	41.8	5.1	0.64	45	0.50	163	4.24
8-2C		62.0	28.8	3.0	0.31	38	0.33	152	3.26
8-1I		70.0	53.2	8.5	0.55	57	0.34	204	2.74
8-2I		65.0	46.1	6.0	0.69	64	0.69	268	6.43
9-1C	15	54.0	47.2	6.2	0.61	54	0.49	140	2.84
9-2C		84.0	56.4	6.9	0.37	52	0.25	216	2.41
9-1I		74.5	43.0	7.2	0.37	77	0.38	250	2.68
9-2I		65.0	50.1	10.0	0.93	41	0.33	163	3.14
10-1C	19	61.0	31.4	3.7	0.33	60	0.50	130	2.39
10-2C		64.5	42.7	5.8	0.40	57	0.36	257	2.70
10-1I		68.0	51.1	6.2	0.34	77	0.40	220	2.51
10-2I		68.5	56.6	7.5	1.04	58	0.74	130	3.72
11-1C	21	51.0	34.0	4.7	0.70	40	0.51	93	2.83
11-2C		98.0	53.5	5.8	0.45	60	0.43	250	4.04
11-1I		62.0	48.5	7.4	0.48	61	0.37	258	3.49
11-2I		76.0	57.0	7.1	0.75	42	0.39	112	2.43
12-1C	23	59.0	25.5	3.4	0.34	44	0.39	160	3.31
12-2C		66.0	40.6	4.4	0.32	53	0.35	300	4.55
12-1I		80.0	64.2	9.5	0.65	6.	0.43	149	2.11
12-2I		61.0	34.6	4.9	0.50	44	0.40	184	3.90
13-1C	27	53.5	22.0	4.3	0.39	43	0.34	247	4.67
13-2C		45.0	29.0	3.6	0.27	48	0.32	292	4.58
13-1I		94.0	63.0	6.7	0.42	63	0.37	278	3.64
13-2I		82.5	53.5	6.7	0.78	52	0.55	154	3.71
14-1C	30	77.0	37.4	4.6	0.42	38	0.30	100	1.87
14-2C		55.0	34.0	4.2	0.40	43	0.36	217	4.30
14-1I		53.0	30.8	3.7	0.44	35	0.35	180	4.44
14-2I		65.0	54.4	8.6	0.86	42	0.37	180	3.73

Stem heights were measured to the nearest half unit.

All values measured per run are obtained from a single plant used.

Experiment conducted December/January 1977-1978.

Table 6. Experiment 6. Resistant Tomato Plants

Experiment Run Number	Time (days)	Stem Height (cm)	Top Weight (g)	Root Weight (g)	Volume of Gas (ml)	C_2H_4 Peak Size (counts)	C_2H_4 (ng/g root)	C_2H_6 Peak Size (counts)	C_2H_6 (ng/g root)
1-1C	0.25	55.0	49.0	7.8	1.58	19	0.25	21	0.78
1-2C		77.0	48.8	7.1	0.51	25	0.13	28	0.38
1-1I		88.0	65.8	14.1	0.87	36	0.19	24	0.28
1-2I		97.5	64.5	11.4	1.72	24	0.27	17	0.45
2-1C	1.25	82.5	54.7	10.8	1.75	25	0.30	22	0.66
2-2C		58.0	35.6	4.3	0.40	26	0.18	31	0.55
2-1I		81.0	71.2	5.2	0.95	26	0.36	27	0.93
2-2I		64.0	40.2	16.7	1.03	31	0.15	25	0.29
3-1C	2	67.5	41.4	6.4	1.11	41	0.62	30	1.00
3-2C		59.5	63.9	16.2	0.76	33	0.13	70	0.67
3-1I		52.0	30.3	5.5	0.45	43	0.31	73	1.21
3-2I		93.0	63.5	15.4	1.93	38	0.41	39	0.72
4-1C	4	64.5	39.1	6.9	1.03	39	0.50	44	1.30
4-2C		58.5	46.7	10.4	0.52	49	0.22	100	1.03
4-1I		61.5	50.3	7.5	0.51	49	0.30	100	1.39
4-2I		59.0	55.9	19.4	2.11	37	0.34	40	0.85
5-1C	7	68.5	38.6	6.4	0.82	53	0.62	119	3.14
5-2C		63.0	34.3	5.2	0.35	47	0.28	140	1.95
5-1I		59.0	68.5	14.6	0.59	63	0.24	214	1.80
5-2I		46.0	36.1	6.0	0.73	44	0.47	101	2.52
6-1C	9	66.0	33.9	7.4	0.69	43	0.36	78	1.48
6-2C		55.5	20.0	5.1	0.33	70	0.43	164	2.20
6-1I		64.0	59.7	13.5	0.84	61	0.35	139	1.79
6-2I		60.0	71.0	14.5	1.62	41	0.40	56	1.25
7-1C	11	57.0	36.5	6.6	0.74	48	0.48	182	4.24
7-2C		70.0	36.6	7.0	0.62	53	0.43	195	3.59
7-1I		91.0	71.5	13.4	0.96	69	0.47	241	3.60
7-2I		41.0	50.5	7.1	1.09	35	0.45	135	4.28
8-1C	13	80.0	50.3	5.0	0.74	27	0.31	113	3.43
8-2C		62.0	44.4	8.8	0.64	36	0.22	135	2.03
8-1I		68.0	64.0	10.8	0.71	61	0.37	240	3.29
8-2I		66.0	73.0	12.8	1.27	48	0.43	192	3.96
9-1C	15	56.5	22.7	3.8	0.69	39	0.61	158	5.94
9-2C		75.0	41.9	5.5	0.31	84	0.46	268	3.15
9-1I		50.0	47.1	8.2	0.46	75	0.40	325	3.81
9-2I		89.0	52.7	11.8	0.87	52	0.35	164	2.51
10-1C	19	48.5	35.6	4.1	0.59	64	0.86	162	4.84
10-2C		54.0	38.0	7.2	0.39	67	0.34	243	2.74
10-1I		79.0	67.5	11.1	0.61	93	0.50	251	2.87
10-2I		77.0	62.9	12.8	1.11	70	0.57	123	2.19
11-1C	21	66.5	41.1	5.7	0.68	38	0.39	114	2.79
11-2C		62.0	23.0	2.7	0.25	61	0.38	273	5.28
11-1I		31.0	32.9	5.4	0.41	81	0.59	268	4.24
11-2I		78.0	65.0	11.2	1.12	36	0.30	111	2.28
12-1C	23	59.0	38	4.3	0.66	31	0.38	110	3.47
12-2C		80.0	43.2	6.6	0.46	75	0.50	285	4.15
12-1I		55.0	40.1	8.9	0.50	62	0.32	260	3.04
12-2I		80.0	63.3	12.8	0.94	48	0.32	151	2.29
13-1C	27	87.0	46.8	7.0	1.60	18	0.26	73	3.38
13-2C		74.0	44.2	6.7	0.50	43	0.28	260	4.04
13-1I		63.0	40.5	9.9	0.55	76	0.40	318	3.69
13-2I		84.0	58.8	10.6	0.93	52	0.41	159	2.89
14-1C	30	64.0	36.9	6.9	0.72	38	0.34	164	3.55
14-2C		66.0	34.1	4.0	0.37	48	0.40	292	5.64
14-1I		63.5	49.9	8.5	0.58	46	0.28	210	2.98
14-2I		69.0	50.8	8.0	0.83	28	0.23	122	2.60

Stem heights were measured to the nearest half unit.

All values measured per run were obtained from a single plant used.

Experiment conducted December/January, 1977-1978.

Appendix II. Standard Curve Raw Data

Table 1. Ethylene Standard Curve Raw Data. Experiments 1-4.

Standard Concentration (μl C_2H_4/l)	Sample Size	Peak Size for Each N (counts)	Mean Peak Size (counts)	Standard Deviation	Limits (95%) of Mean Upper (counts)	Lower (counts)
0.044	13	1.9, 5.6, 4.3, 11.6, 14.2, 3.7 4.8, 9.0, 8.3, 10.7, 11.5, 8.8 8.3	7.9	3.63	10.1	5.7
0.13	18	3.8, 8.5, 5,0, 12.2, 12.0, 9.6, 10.5, 6.3, 7.5, 7.5, 12.0, 11.0, 9.5, 12.0 24.0, 9.0, 6.0, 10.5, 22.5	10.7	5.23	13.3	8.1
0.26	12	12.7, 13.0, 13.0, 13.0, 11.5, 9.4, 6, 8.8, 13.5, 15.5, 14.0, 15.4	12.2	2.81	14.0	10.4
0.45	15	9.5, 12.8, 6.0, 11.2, 12.2, 11.3, 26.2, 22.5, 10.3, 13.0, 14.0, 12.5, 7.5, 9.0, 20.5	13.1	5.81	16.3	9.9
0.63	11	9.9, 13.8, 22.6, 23.5, 22.3 29.0, 26.5, 28.3, 24.3, 13.0 22.0	21.4	6.38	25.7	17.1
1.11	13	24.1, 19.5, 31.0, 23.7, 27.0, 51.3, 48.0, 34.7, 21.0, 56.0, 28.5, 13.0, 21.0	30.7	13.3	38.7	22.7
2.25	11	34.7, 30.2, 35.8, 42.7, 38.0, 39.7, 34.5, 31.0, 34.5, 35.5 34.0	35.5	3.59	37.9	33.1
4.43	9	70.3, 65.1, 66.2, 75.5, 70.0, 70.0, 64.0	68.6	3.47	71.3	65.9

Table 1.

Confidence limits about the mean were calculated from the formula $\bar{X} \pm t(s/\sqrt{n})$ as outlined in Methods Section 4-10a.

Mean Barometric Pressure = 752.6 mm Hg

Mean Temperature = 23.3°C

Table 2. Ethylene Standard Curve Raw Data. Experiments 5, 6.

Standard Concentration (μl C_2H_4/l)	Sample Size	Peak Size for Each N (counts)	Mean Peak Size (counts)	Standard Deviation	Limits (95%) of Mean Upper (counts)	Lower (counts)
0.045	13	7.0, 7.5, 12.0, 8.0, 7.5, 7.5, 6.0, 8.5, 8.5, 5.0, 8.5, 13.0	8.3	2.16	9.6	6.8
0.63	23	23.0, 22.0, 16.0, 15.5, 15.0, 18.5, 16.5, 11.0, 14.5, 15.5, 29.0, 23.5, 16.5, 18.0, 15.5, 13.0, 11.0, 28.0, 11.0, 9.0, 16.0, 18.0, 14.0, 19.0	16.8	5.03	19.0	14.6
1.11	21	17.0, 16.5, 14.0, 18.0, 20.5, 20.5, 11.5, 12.0, 12.0, 17.0, 16.5, 9.5, 22.5, 43.1, 33,0, 32.0, 12.0, 32.0, 21.0, 20.0, 19.0	20.0	8.53	23.9	16.1
2.25	15	44.0, 38.0, 36.0, 33.0, 31.5, 30.0 29.0, 31.5, 28.5, 43.5, 40.5, 32.0, 37.0, 37.0, 35.0	35.1	4.94	37.9	32.4
4.43	15	58.0, 55.5, 53.0, 66.0, 69.0, 63.0, 36.0, 34.5, 37.5, 52.5, 60.0, 52.5, 53.0, 54.0, 52.5	53.1	10.3	58.8	47.4
9.57	9	114.0, 108.0, 110.0, 89.0, 109.0, 99.0, 116.0, 113.0, 150.0	112	16.6	125.0	99.0

Table 2.

Confidence limits about the mean were calculated from the formula $\bar{X} \pm t(s/\sqrt{n})$ as outlined in Methods Section 4-10a.

Peak sizes were measured to the nearest half unit.

Mean Barometric Pressure = 755.3

Mean Temperature = 22.1°C

Table 3. Ethane Standard Curve Raw Data. Experiments 1-4.

Standard Concentration (μl C_2H_6/l)	Sample Size	Peak Size for Each N (counts)	Mean Peak Size (counts)	Standard Deviation	Limits (95%) of Mean	
					Upper (counts)	Lower (counts)
0.13	11	4.5, 4.0, 4.5, 3.0, 5.0, 5.5, 3.5, 3.5, 2.0, 2.0, 2.0, 3.5	3.7	1.13	4.5	3.0
0.45	13	11.0, 9.0, 13.0, 10.0, 8.0, 4.0 5.0, 6.0, 4.0, 7.0, 5.5	7.7	2.77	9.4	6.0
0.63	14	14.5, 16.0, 18.0, 16.0, 6.0, 11.0, 8.5, 12.0, 8.0, 7.0, 6.0, 4.0, 7.0, 5.5	10.9	3.95	13.2	8.6
1.11	12	25.0, 27.5, 48.0, 28.0, 14.5, 14.0, 14.0, 12.5, 15.0, 15.0 19.0, 14.0	20.1	12.8	28.2	12.0
2.25	14	28.0, 55.5, 32.0, 26.0, 34.0, 34.0, 29.0, 23.0, 22.5, 21.5, 21.0, 35.0, 29.0, 23.0	29.5	8.93	34.7	24.3
4.43	13	34.0, 33.0, 31.0, 33.0, 42.5, 36.5, 32.5, 51.0, 37.0, 44.0, 31.0, 78.0, 49.0	41.0	13.0	48.9	33.1

Confidence limits about the mean were calculated from the formula $\bar{X} \pm t(s/\sqrt{n})$ as outlined in Methods Section 4-10a.

Peak sizes were measured to the nearest half unit.

Mean barometric pressure = 755.2 mm Hg.

Mean temperature = 23.6°C

Table 4. Ethane Standard Curve Raw Data. Experiments 5, 6.

Standard Concentration (μl C_2H_6/l)	Sample Size	Peak Size for Each N (counts)	Mean Peak Size (counts)	Standard Deviation	Limits (95%) of Mean	
					Upper (counts)	Lower (counts)
0.63	9	5.0, 3.0, 5.0, 5.0, 5.5, 5.0, 9.0, 11.0, 6.0	5.9	2.43	7.8	4.0
1.11	9	10.0, 13.0, 10.0, 8.0, 10.0, 11.0, 18.0, 11.0, 12.5	11.5	2.85	13.7	9.3
2.32	9	15.0, 21.0, 8.0, 15.0, 16.0, 14.0, 33.0, 17.0, 24.0	18.1	7.15	23.6	12.6
4.43	9	26.0, 25.0, 13.0, 33.5, 28.5, 35.0, 25.0, 25.0, 27.0	26.4	6.25	31.2	21.6
9.57	11	52.0, 41.0, 53.0, 60.0, 45.0, 54.0, 71.0, 63.0, 60.0, 61.0, 44.0	54.9	9.15	61.5	48.3
15.2	12	101.0, 116.0, 121.0, 91.0, 92.0, 93.0, 83.0, 83.0, 88.0, 87.0, 85.0, 90.0	94.2	12.4	102.0	86.3

Confidence limits about the mean were calculated from the formula $\bar{X} \pm t(s/\sqrt{n})$ as outlined in Methods Section 4-10a.

Peak sizes were measured to the nearest half unit.

Mean Barometric pressure = 755.6 mm Hg

Mean Temperature = 23.1°C