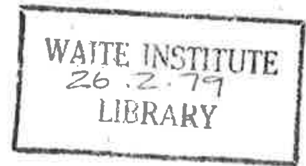

THE PHYSIOLOGY OF TOMATO PLANTS INFECTED WITH ROOT-KNOT NEMATODE, *MELOIDOGYNE JAVANICA*

by

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TABLE OF CONTENTS

		Page
DECLARATION		(iii)
SUMMARY		1
CHAPTER I	INTRODUCTION	3
CHAPTER II	LITERATURE REVIEW	5
CHAPTER III	EFFECTS OF DIFFERENT CONDITIONS ON GROWTH OF TOMATO PLANTS INFECTED WITH *M. JAVANICA*	18
	1. No Stress	19
	2. Effect of Temperature	20
	3. Effect of Soil Moisture	21
	4. Effect of Nutrients	21
	5. Discussion	29
CHAPTER IV	INFLUENCE OF *M. JAVANICA* ON THE PHYSIOLOGY OF TOMATO PLANTS	32
	1. Histological Studies	33
	2. Measurements of Root Resistance by 'Bomb Calorimeter'	36
	3. Mineral Content and Distribution	41
	4. Water Relations	43
	(a) Diffusive Resistance of Stomata	46
	(b) Leaf Water Potential	47
	(c) Transpiration	47
	5. Hormonal Imbalance	52
	(a) Gibberellins	54
	(b) Cytokinins	55
	(c) Abscissic Acid	55
	6. Discussion	62

Page

CHAPTER V EFFECT OF INFECTION ON AMINO-ACID COMPOSITION 69

1. Free and Hydrolysed Amino Acid Composition 69
2. Proline Accumulation 74
3. Effect of Exogenous Amino Acid and Antimetabolites on Growth of *M. javanica* and Tomato Plants 82
4. Discussion 90

CHAPTER VI GENERAL DISCUSSION AND CONCLUSIONS 97

ACKNOWLEDGEMENTS 100

BIBLIOGRAPHY 101

PUBLICATIONS 113

DECLARATION

This thesis has not previously been submitted for an academic award at this or any other University, and is the original work of the author, except where due reference is made in the text.

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SUMMARY

The purpose of this research was to study the influence of infection by *Meloidogyne javanica* on the growth and physiology of tomato plants. The research is, therefore, mainly concerned with the pathology of the host plant in an attempt to ascertain the importance of some of the physiological components of the plant and how they affect plant growth and nematode populations.

Initial experiments assessed the effect of different levels of nutrients, temperature and soil water and interactions between these factors and inoculum density of nematodes on plant growth. Although root weights increased with increase in the density of inoculum, due to galling, there was little indication that the nematodes influenced top growth at different temperatures or soil water levels. Interaction occurred between levels of soil nutrients and initial population density of nematodes on plant growth. In general top growth was maintained in spite of nematode infection. How growth was maintained and how the nematode damaged the plant were then studied.

Histological studies by light and scanning electron microscopy indicated that the xylem vessels close to nematodes in the roots were disrupted. Giant cells formed in the provascular region even before the xylem pattern was established. The development of giant cells from the parenchyma cells inhibited cambium production, consequently no secondary xylem was formed. Abnormal xylem was not arranged longitudinally but was dispersed in a diffuse and disconnected manner. Thus the efficiency of the xylem as a conducting system was probably impaired. Measurements of the resistance of infected roots to water

flow and of water potential in the leaves supported this hypothesis. Nevertheless plants did not wilt and were not stunted. Measurements of stomatal diffusivity indicated that at the same water potentials, stomata in leaves of infected plants had a higher diffusive resistance than those in uninfected plants. Here is one mechanism that appears to maintain the integrity of the infected host plant by conserving water. Measurements of gibberellic aicd, cytokinins and abscissic acid suggested that the enhanced stomatal regulation in infected plants might be controlled by such hormones.

Studies on the amino acid composition of infected and uninfected plants indicated that total free amino acids, particularly proline and its precursors, increased in the infected roots. Higher concentrations of proline occurred in roots during the initiation of egg laying suggesting that proline was required for the production of eggs. High proline concentrations in eggs, egg sacs, females and galls supported this. Such studies suggested that a metabolic sink was created at sites in the roots where nematodes had formed giant cells and were reproducing. The possible significance of proline in the nematode-plant relationship and its use as a means of nematode control are discussed.

CHAPTER I

INTRODUCTION

The purpose of this research was to study the influence of infection by *Meloidogyne javanica* (Treub,) Chitwood on the growth and physiology of tomato (*Lycopersicum esculentum* Mill. cv. Early Dwarf Red) plants. *M. javanica* is a widespread pathogen, attacks a large number of plant species, and is known to have caused serious losses in crops especially when its numbers reach high levels and growth conditions are marginal.

This study is concerned with plant pathological aspects; in particularly, it aims to devise methods whereby the data obtained can be used, after statistical analysis, to assess the relative importance of the various physiological components studied, on the growth of plants and on nematode populations. Such information is, of course, relevant in designing a control programme for the nematodes.

There is an extensive literature on the detrimental influence of root-knot nematodes on crop yield. This could be due to the fact that infected roots are grossly distorted and thus infection is very obvious. In fact, root-knot is one of the oldest known nematological disorders of plants; Berkeley (1855) described it from the United Kingdom on cucumbers. Hence, more study has been assigned to this problem than to the more insidious debilitating effects on plant yield that some other plant parasitic nematodes cause. Numerous papers have reported on the host-relationship and on histopathology studies, but less attention has been paid to experimental study in the intact plant of factors such as water status, hormonal imbalance and biochemical changes.

To emphasize these points and to set the stage for the description of experiments that follow, a brief review of the published literature relevant to the physiology of plants infected by root-knot nematodes will be given.

CHAPTER II

LITERATURE REVIEW

Root-knot nematodes (*Meloidogyne* spp.) have a very wide host range, including both crop plants and weeds (Bird, 1974). Generally, above ground symptoms are similar to those caused by many root diseases or environmental factors. In the field, infections are usually associated with loss in yield, stunting and reduced rate of growth, root impairment, increased wilting, mineral deficiencies, discolouration of foliage, twisted leaves and distorted shoots. Normally plants linger through the growing period and are seldom killed prematurely. Formation of galls on the roots and devitalising of root tips might not only deprive plants of nutrients but also cause disruption of the vascular system. These responses are related to the number of second-stage infective larvae entering and becoming established within the plant. Only at a very high inoculum level is the growth of infected plants significantly reduced when compared with controls and with plants infected with fewer larvae of *Meloidogyne* (Bird, 1970).

These responses are all indicative of a lack of physiological stability, that we call - disease. They may be caused by any one of many changes in physiological function. It is often difficult to prove conclusively whether nematodes, other microorganisms, other limiting factors or combinations of these are the cause of the root impairment. Stunting and poor growth may be caused by reduced translocation, inadequate nutrient absorption, abnormal production of growth regulators, reduction in the number of roots, toxic metabolites

and so on. If the physiology of the diseased plant was studied in detail, it would probably be found that any one symptom was the result of numerous interacting factors. Differences in plant response to parasitism by *Meloidogyne* can be the result of an interaction between a number of physiological processes in the host itself. Clearly plants growing rapidly in a suitable environment will do better than those struggling in a poor environment and exposed to the same number of nematodes. Reactions of the whole plant to nematode infection do not usually give a good indication of the kind of biochemical and tissue disturbances that have occurred. In describing the syndromes of diseased plants, the effects and not the causes of malfunction are considered. Such effects are often measures of malfunction, however, so they are important in this context.

Over the years most physiological and biochemical measurements made on host plant responses to *Meloidogyne* have been made on dissected, extracted or homogenised infected material simply because this has been the most practical way to obtain material for these measurements. Much information has been obtained using these methods, particularly with regard to giant cells - their nature and development. Several types of physiological measurements have been made on whole, infected plants. These experiments are divided rather loosely into two categories: first, those in which various types of specific activators or inhibitors of physiological processes are applied to infected plants and the growth rates or reproductive rates of the nematodes in these plants are compared with similar rates in untreated infected plants, and secondly, measurement of normal physiological function in infected plants and uninfected controls. Physiological measurements on the responses

of whole, intact plants susceptible to *Meloidogyne* have been made only recently. This is probably a reflection of the availability of apparatus for these types of measurements and the degree of co-operation that has existed between plant nematologists and plant physiologists (Loveys and Bird, 1973).

Nematode numbers, soil moisture, temperature, nutrients and plant age influence the rate of invasion and reproduction of *Meloidogyne* in tomato roots and the growth of the host plant (Wallace, 1969, 1970). Severe crop losses in the field may be indicative of an interaction between nematode numbers and some environmental factors causing stress to the plant. In most cases, however, it is difficult, without ecological studies to assess whether nematodes are the main cause of reduction in plant growth as many other factors may be of equal importance.

Wallace (1969) indicated that the effect of nematodes on plants could be usefully considered as an ecological problem involving interactions between environment, nematodes and the plants. Further work (Wallace, 1970), suggested the presence of some interactions between initial population density of nematodes and some environmental factors (soil nutrients, water, etc.) on the growth of the tomato plants. The occurrence of interactions suggests that in the field, stressful conditions may occur where the presence of nematodes causes marked reductions in plant growth. Hence in a well-fertilised soil a plant may tolerate a particular population level but in an infertile soil the same population might cause marked reductions in growth and yield.

Although species of *Meloidogyne* are today well-known plant pathogens, information on their host-parasite relationships is still fragmentary. Observations have been made on what are fairly obvious

morphological symptoms. Most species have been reported to cause galling of roots, and the histology of such galls has been well investigated. The ultrastructure and histochemistry of giant cells induced by *Meloidogyne* in tomato have been studied and reviewed by Bird (1961). These structures were first studied by Treub (1886) 75 years ago, and have been investigated periodically since then (Molliard, 1900; Tischler, 1901; Nemec, 1910; Kostoff and Kendall, 1930; Christie, 1936; Cole and Howard, 1958; Crittendon, 1958; Krusberg and Nielsen, 1958; Mildenberger and Wartenberg, 1958; Davis and Jenkins, 1960; Owens and Novotny, 1960). These were reviewed in detail by Webster (1969) and have since been elaborated upon (Huang and Maggenti, 1969a and b; Paulson and Webster, 1969, 1970; Bird, 1972a and b; Jones and Northcote, 1972) in order to elucidate some of the problems associated with the method of wall formation, nuclear origin and the mode of formation and function of the giant cell.

In view of the volume of literature being published on this topic, it is suprising that most studies on the histopathological changes in infected roots are mainly concerned with giant cell formation. Recent workers on the other hand, while working with the galls produced by *Meloidogyne javanica* (Treub,) Chitwood casually noticed the occurrence of abnormal xylem-like elements (Christie, 1936; Krusberg and Nielsen, 1958; Siddiqui and Taylor, 1970). Similar patterns of xylem production were also reported by Epstein and Cohn (1971) on *Coleus* segments infected with *Longidorus africanus* Morny in culture. A survey of literature reveals that only a few attempts have been made so far to study in detail the histopathology of roots infected by *Meloidogyne* with regard to atypical xylem formation (Swamy and Krishnamurthy, 1971; Farooq, 1973). Siddiqui *et al*. (1974) studied the development of abnormal xylem due to *Meloidogyne incognita*

Kofoid and White in the roots of *Lagenaria leucantha* L.

In fact, the nature and extent of the injury caused by galling is not well defined. The symptoms associated with these types of diseases suggest, as one of the causes, some disturbance of, or interference with, the sap flow in the vascular system. This relationship between galls and interference to sap flow was first studied in crown gall (*Agrobacterium tumefaciens*) on young apple trees and tomato plants (Melhus, Muncie and Ho, 1924). Again, this phenomenon of impedence to water flow and wilting incidental to the wilt diseases was studied in cabbage yellows (*Fusarium conglutinans*), and in alfalfa wilt (causal agent unknown) (Melhus, Muncie and Ho, 1924). The interference to flow in plants affected with these diseases, was measured using a special piece of apparatus designated as a "Bomb calorimeter". There was a difference in the rate of flow through galled and healthy plants and between the various levels of infection, and this gives an indication of the extent of interference by galls in the infected roots.

Wallace (1974) in his studies on photosynthesis and carbon dioxide incorporation into infected and uninfected tomato plants by *M. javanica* indicated that the creation of so-called metabolic sinks in the roots, caused by formation of giant cells, was unlikely to be the main cause of nutrient deprivation in the roots. He further suggested that the photosynthetic data indicated that inhibition of upward translocation of water and nutrients may be more important. Loveys and Bird (1973), for example, reported that heavy invasion of roots by nematode larvae led to a change in the supply of root-derived photosynthetic regulating factors. But the physiological nature of these factors was not determined. Root damage could also lead to water

stress in the plant and cause partial closure of the stomata which would then result in a difference in the rate of carbon dioxide fixation. Although neither infected nor control plants showed signs of wilting in this study, this mechanism of inhibition of photosynthesis must be investigated as an alternative.

Internal water stress due to increasing resistance to water uptake or translocation within the infected plants influences many physiological processes adversely. This results in growth inhibition in herbaceous plants (Woodhams and Kozlowski, 1954; Kramer, 1963) and woody plants (Kozlowski, 1964). Voluminous data are available showing that water stress affects water uptake, root pressure, seed germination, stomatal closure, transpiration, photosynthesis, enzymatic activity, mineral relations, nitrogen metabolism and other processes (Kramer, 1949; Army and Kozlowski, 1951; Kozlowski, 1964). Increases in stomatal resistance have been reported by MacHardy *et al.* (1976) in *Verticillium* wilt of chrysanthemum, Duniway (1975) in *Phytophthora* root rot of safflower and Roberts *et al.* (1977) in Dutch Elm disease of American Elm. Most studies have been centred on fungal wilt diseases and data on the effect of nematodes on the water-relations of the infected plants are scarce. However, Kaplan *et al.* (1976) showed that sunflower plants infected with *Pratylenchus penetrans* (Cobb) Filip. and Stek. have higher diffusive resistance regardless of age than healthy plants growing under similar conditions. He further suggested that such moisture stress may induce the stunting commonly associated with nematode infection.

The exact manner by which internal water stress affects various aspects of growth has been a subject of vigorous debate. There is evidence that internal water stress affects growth by both direct and indirect mechanisms, with the latter operating by causing hormonal and

mineral imbalance. Itai and Vaadia (1971) reported that water stress applied to the shoot through enhanced evaporative demand reduced cytokinin activity in extracts of xylem exudate and leaves. This reduction resembled the changes in cytokinin activity caused by water stress applied to the root. Ref? There is no aspect of host-parasite interactions of plant-parasitic nematodes that has been examined more extensively than that of the associated changes in plant hormones. This is understandable when one considers the essential roles that the various hormones play in the normal growth of the plant, bearing in mind that the presence of a nematode and its secretions invariably modifies plant growth. Nevertheless, the lack of progress in this area of research is almost inevitable as the plant physiologists themselves do not know precisely the mode of action of many of the hormones in plant growth.

In the last few years, many authors have found that in the above-ground parts of plants directly attacked by parasites, there is a rise in the level of free cytokinins (Pozsar and Kiraly, 1966; Kiraly *et al.*, 1967; Reddy and Williams, 1970; Vizarova, 1971). An increase of cytokinin activity in roots was mentioned only in the case of roots that were directly attacked by some parasite, as for instance when *Brassica rapa* L. was infected with *Plasmodiophora* (Matsbura Nakhira, 1968). Since then, increasing attention has been focused on the role of growth regulators in the physiology of the diseased plants. Goodman, Kiraly and Zaitlin (1967) reviewed the significance of indole acetic acid in crown gall disease of tomato and gibberellins in Bakanae disease of rice. Plums infected with the red spider mite showed an increase in gibberellin-like substances (Avery and Lacey, 1968).

Plant growth hormones play a role in cytokinesis, cell hypertrophy and in the synthesis of DNA, RNA and protein, and consequently they are involved in plant tissue responses such as giant cell formation (Viglierchio, 1971).

In examining the plant hormones associated with various species of nematode infecting different plant species, Viglierchio and Yu (1968) found that the kind of auxin present in the nematode-infected tissue depended on the species of nematode present. Thus, in Brussel sprouts infected by *M. incognita* indole butyric acid appeared in the tissue but there was a reduction in indole-acetonitrite (IAN) while the proportions of indole acetic acid (IAA) and indole acetic acid ethyl ester (IAE) remained the same as in healthy tissue. In extracts of Brussel sprouts infected with *M. javanica*, however, there was no IAE present, IAA increased and IAN remained the same as in the healthy plant. It appears, therefore, that the type and, to some extent, the relative concentrations of auxin present in nematode infected tissue are primarily a characteristic of the nematode species but can be moderated to some extent by the host. Indole compounds were detected by Balasubramaniam and Rangaswami (1962) in root galls induced by *M. javanica*. A higher level of endogenous auxins (Cutler and Krusberg, 1968) or of auxins and cytokinins (Kochba and Samish, 1972) or of exogenously applied cytokinin (Dropkin *et al.*, 1969; Kochba and Samish, 1971) in infected roots is associated with the production of giant cells and galls. Wick applications of naphthalene acetic acid (NAA) and kinetin together enhanced development of *M. javanica* in both susceptible and resistant young peach seedlings (Kochba and Samish, 1971). The fact that endogenous levels of cytokinin were greater in susceptible than in resistant peach roots (Kochba and Samish, 1972) supports the hypothesis that cytokinin may be a key controlling factor in the

development of giant cells, especially as cytokinins are recognised regulators of cell enlargement, karyokinesis and DNA synthesis.

The gibberellin content of shoots from citrus trees infected with *Radopholus similis* (Cobb.) Thorne declined when compared with the gibberellin content of shoots from non-infected trees (Hanks and Feldman, 1968). Similarly, infection of tomato by *M. incognita* decreased the production of neutral and acidic gibberellins and decreased also the cytokinin levels (Brueske and Bergeson, 1972). Such changes in gibberellins production probably result in decreased translocation of gibberellins, which may be one of the causes of stunted growth. The authors also speculate that wilting of the diseased plant would be caused by decreased gibberellin and cytokinin in the xylem.

The evidence to date suggests that plants are more susceptible to nematode attack in the presence of higher levels of some plant hormones. Whatever the specific role of plant hormones in facilitating a susceptible response of the plant to the nematode, the type of tissue response can be related to the known action of auxin in changing enzyme activity and increasing amino acid and water uptake of cells.

In recent years, there has been increasingly more emphasis on the use of biochemical methods and techniques for research in plant pathology and it has now become possible to offer biochemical explanations for several phytopathological phenomena, particularly on the mechanisms involved in the invasion of plants by pathogens, the production of disease symptoms, and the mechanisms involved in the resistance of plants to the invading microorganisms. Thus a recent trend in characterizing plant diseases is based on some aspect of the biochemistry of the host-parasite relationship. Changes in amino acid and amide composition as affected by virus infection (Fife, 1956; Diener, 1960; Selman,

Bierley, Pegg and Hill, 1961), by fungal infection (Benedict and Hilderbrand, 1958; Burton and deZeeuw, 1961; Shaw and Colotelo, 1961), by nutrient deficiency (Steinberg, 1956; Freney, Delwiche and Johnson, 1959), and by nematodes (Myuge, 1956; Owens and Novotny, 1960; Krusberg, 1961; Hanks and Feldman, 1963; Feldman and Hanks, 1964; Saxena, 1972) in plants have received attention. Only a few of these studies present quantitative data.

Associated with these changes in amino acid composition, characteristic accumulation in the levels of free proline was noted. Proline may also accumulate under certain other stress conditions, such as wilting (Barnett and Naylor, 1966; Chen, Kessler and Monselise, 1964; Kemble and Macpherson, 1954; Prusakova, 1960), nutrient deficiency (Seitz and Hochter, 1964) or disease (Thompson *et al.*, 1960). Owens and Specht (1966) reported that galls induced by *M. incognita* in tomato contained higher levels of amino acids (particularly proline) and proteins. The outstanding feature of roots of *Bidens tripartita* parasitized by *Longidorus africanus* was the large amount of free proline they contained (Epstein and Cohn, 1971). Free proline in substantial quantities was also reported after infection by *Radopholus similis* in grapefruit seedlings (Hanks and Feldman, 1963) and by *Meloidogyne* in tomato (Owens and Specht, 1966), in alfalfa tissue galled by *Agrobacterium tumefaciens* (Smith and Townsend) Conn. (Seitz and Hochter, 1964), in water deficient wheat (Gusev and Gordon, 1968), ladino clover (Routley, 1966) and Bermuda grass (Barnett and Naylor, 1966). Other workers have indicated that proline may be incorporated in high amounts in egg shells of nematodes. Clarke *et al.* (1967) found that free proline comprises 38.3% of the total amino acids of 24-hour hydrolysates from egg shells of *Heterodera rostochiensis* Wollenweber. The proline in the egg shells of *M. javanica* was thought

to be incorporated into the shells during the later stages of their development (Bird and McClure, 1976).

Coleus stem segments treated with proline after pretreatments with IAA showed increased formation of xylem elements (Roberts and Baba, 1968). They suggested that proline might act as a "xylogenic factor" released by the ruptured vascular bundles when an intact plant is wounded. Cohn and Orion (1970) found xylem elements scattered in galled tissues from *Longidorus africanus* infected plants. These observations, coupled with the findings of Epstein and Cohn (1971) that roots of grape seedlings and burr marigold parasitized by *Longidorus africanus* also contain more proline support the contention that proline might play a role in xylogenesis.

The simultaneous decrease of aspartic acid and increase of proline in roots of *Bidens tripartita* parasitized by *Longidorus africanus* (Epstein and Cohn, 1971) suggest a correlation between them. Work on the metabolism of radioactive proline in leaves has shown that proline was converted to asparagine and glutamine (Steward and Bidwell, 1962) or to aspartic acid and glutamic acid probably via the tricarboxylic acid cycle (Wang, 1968). Steward *et al.* (1966) similarly explained the accumulation of proline in wilted leaves, postulating a net synthesis from sugars via glutamic acid, and that proline serves as a readily available storage compound in the stressed plants.

Growth hormones were also involved in the accumulation of proline in stressed plants. Generally, cytokinin decline in stressed plants (Itai and Vaadia, 1971; Burrows and Carr, 1969) and abscissic acid increases (Wright and Hiron, 1969; Milborrow and Noodle, 1970; Mizrahi *et al.*, 1970). In addition, Railton and Reid (1973) have shown that application of exogenous cytokinin to flooded plants can

relieve most of the symptoms of flooding injury. Spraying the foliage of sunflower with benzyladenine and abscissic acid respectively and subjecting them to stress showed no significant increase in endogenous proline. In the case of abscissic acid, it could be taken as circumstantial evidence against the idea that increases in abscissic acid associated with stress are directly related to the observed proline accumulation.

A reduction in endogenous cytokinin as a result of wilting could in some way affect amino acid or protein metabolism and allow for an increase in free proline. In view of the enormous amount of data, it is surprising that the nature and cause of proline accumulation under stress is still contradictory. An interesting topic for future research might be to determine how these factors (hormones, pathogens, ect.) modify protein synthesis and the proline pool in plants under stress and the possibility of incorporating proline or its analogues in counteracting the response to infection.

The ultimate resolution of nematode control may lie in the development of chemical analogues which may serve as antimetabolites in the biological systems of the nematode without seriously interfering with the normal metabolism of the host. Such chemicals, due to their biological specificity, would block specific metabolic reactions and cause the nematode to "starve" in the midst of plenty (Overman and Woltz, 1962). The use of structural variants (analogues) of natural metabolites of plants and animals to control populations of undesired species has been poorly developed in the field of agriculture. The effects of certain amino acid analogues upon tomato and other plants were described by Woltz and Jackson (1961). Woltz (1963)

further explored the effects of amino acid antimetabolites on chrysanthemum and reported experiments that demonstrated the nature of the metabolite-antimetabolite phenomenon of the plant. Peacock (1960) attempted to disrupt the normal host-parasite relation of tomato roots and *M. incognita* by "grossly increasing the amino acid content" of the substrate but concluded that the presence of luxury amounts of amino-acid did not affect nematode development.

The fact that amino acids have chemotherapeutic effects on some diseased plants (Van Andel, 1958) and accumulate in the giant cells of nematode infected plants (Owens and Novotny, 1960; Peacock, 1966), suggests that they might be used as nematicides. But studies on the effect of amino acids against plant parasitic nematodes have been generally limited (Overman and Woltz, 1962; Prasad and Webster, 1967; Rao and Prasad, 1969; Evans and Trudgill, 1971; Reddy, Govindu and Setty, 1975a and b).

The advances made in plant physiology and plant pathology in recent years may therefore indicate the direction along which the plant nematologist should travel in the study of malfunctions in the plant. The fact that nematodes and fungi are widely different organisms is less important than the fact that plants often respond in similar ways to both pathogens.

These studies raise numerous questions, but of these the following stand out: How do nematodes damage plants? What is the relative importance of mechanical damage and physiological damage? What biochemical events occur when the nematode penetrates the cell? What effect has nematode damage on the growth of the plant? Do dynamic defense factors occur in plants in response to nematode attack? Experiments described in this thesis attempt to answer these questions.

CHAPTER III

EFFECTS OF DIFFERENT CONDITIONS ON THE GROWTH OF PLANTS INFECTED WITH *M. JAVANICA*

The association of nematode, plant and environment can be considered as a biologic system in which a change in the behaviour or physiology of one of the organisms tends to produce an appropriate change in the other to ensure survival. The extent to which equilibrium is achieved determines the ability of both the plant and nematode to survive, and in the present context the emphasis is on the plant.

Much progress has been made during the last twenty years in characterizing the relationships between nematode densities and plant yield and growth. Damage is largely determined by preplant densities, which is the result of nematodes having limited mobility and low reproductive potentials. This relation can be altered in perennials by re-introduction, build-up of negligible initial densities or both. Crop nematode relationships vary with cultivars, nematode species and races, and environment. Very low population densities of certain nematodes may stimulate plant growth.

Plant parasitic nematodes and host plants are very sensitive to the environment (Sayre, 1971; Wallace, 1971). Environmental stress, by lowering the tolerance of the plant to infection, may be the basis of some of the more serious crop failures due to nematodes (Wallace, 1969). Most above and below ground factors that affect growth of plants as well as other soil microorganisms also influence nematode activity and alter the relationship between numbers of nematodes and crop yield (Wallace, 1969 and 1971).

Malfunction in the infected plant is often increased when the plant grows in a suboptimal environment. This statement can be criticised in two ways: first, increased malfunction does not necessarily imply that defense against nematode attack is less effective. A healthy and unhealthy plant may suffer equal root damage from the same number of nematodes, but because of its prolific root system the healthy plant can tolerate more damage without its growth above ground being affected. Second, there is little reliable evidence to substantiate the statement that malfunction is increased in a suboptimal environment. For example, it is not sufficient to compare the decrease in growth between infected and non-infected plants at two levels of a particular nutritional element, such as nitrogen. All we can say is that both nematodes and shortage of nitrogen inhibit growth. What we really want to know is whether nitrogen deficiency and nematode infection act independently or is there an interaction? The interaction of nematode and host nutrition may depend on nematode density, as with *M. javanica* on tomato (Bird, 1970). Experiments along these lines may help to explain the rather conflicting data on the effect of nutritional and other physiological conditions on nematode growth and plant vigour (Kirkpatrick *et al*., 1964), and these then form the basis for the following studies.

Materials and Methods

1. No stress

Seedlings of tomato were selected for uniformity at the beginning of the experiment and grown in 10 cm pots of John Innes potting compost for 40 days when 1 to 4 day-old larvae of *M. javanica* were introduced on to the surface of the soil at the base of the plant.

The plants were watered daily and randomised on the glasshouse benches with temperatures fluctuating between 20°C and 30°C. Nematode densities were 0, 500, 1,000, 2,000, 5,000 and 10,000 per pot with four replicates per treatment. Thirty-five days after inoculation the plants were removed from the pots and the roots washed free of soil. Fresh weight of roots and tops were obtained as Wallace (1970) has shown that there was no loss in precision or change in relationships if fresh weights were determined instead of dry weights. *M. javanica* reproduced in all plants but no attempts were made to measure the degree of egg production. However, the number of mature females in the roots was assessed by staining in boiling lactophenol cotton blue, clearing in lactophenol and macerating.

2. <u>Effect of soil temperature</u>

To study the effect of soil temperature, twenty-four tomato plants grown in 10 cm pots were placed in waterproof glazed pots in each of three constant temperature tanks. The temperature of these tanks was set at 15°C, 25°C or 35°C. The variation within each temperature level was ±2°C. Four plants from each temperature treatment were inoculated with 0, 500, 1,000, 2,000, 5,000 and 10,000 larvae of *M. javanica*. In order to obtain sufficient infection the temperature in the 15°C and 35°C tanks was initially set at 20°C and 25°C respectively. Three days after inoculation, the temperature was reset at 15°C and 35°C.

At termination of the experiment, fresh weights of tops

and roots were determined, and number of mature females in the galls assessed.

3. Effect of soil moisture content

To study the effect of water stress, the moisture content of the soil used was adjusted to give a moisture content of 8%, 16% and 25%. This was done by initially weighing the soil, pot and plant together and adding water to bring the total weight of the system to its required moisture content. The moisture content was kept constant by adding water to constant weight daily. Twenty-four plants were used for each moisture treatment and four plants from each treatment group were inoculated with 0, 500, 1,000, 2,000, 5,000 and 10,000 larvae of *M. javanica*. In order to obtain sufficient infection, the moisture level in the 8% and 25% range was initially set at 16%. Three days after inoculation the moisture content was reset to 8% and 25%. Pots were randomised in the growth chamber with temperatures between 20° - 25°C and twelve hours of light. Determination of fresh weight of tops and roots and number of females were as described in the previous experiments.

4. Effect of soil nutrients

The experiment was conducted on tomato seedlings grown in plastic tubes (diameter 3 cm x 15 cm in length) filled with sand. During the first twenty days the seedlings were supplied with complete nutrient solution (Hoagland and Arnold, 1950) once every four days. After twenty days they were subjected to various mineral element deficiency treatments. The composition of the nutrients used was

as follows:

Composition of nutrient solution used in studying the effect of host nutrition and inoculum levels on the growth of tomato plants (ml per litre)

Elements	NPK	-N	-P	-K	-NP	-NK	-PK	-NPK
1M KH_2PO_4	1	-	-	-	-	-	-	-
1M KNO_3	5	-	6	-	-	-	-	-
1M $Ca(NO_3)_2$	5	-	4	5	-	-	5	-
1M $MgSO_4$	2	2	2	2	2	2	2	2
0.5M K_2SO_4	-	5	-	-	-	-	-	-
0.5M $Ca(H_2PO_4)_2$	-	10	-	10	-	10	-	-
0.01 $CaSO_4.2H_2O$	-	200	-	-	200	-	-	200
Trace elements	1	1	1	1	1	1	1	1

One litre of stock solution for the trace elements, contained H_3BO_3, 2.8 g; $MnCl_2.4H_2O$, 1.81 g; $Zn.SO_4.7H_2O$, 0.22 g; $CuSO_4.5H_2O$, 0.08 g; $H_2Mo\ Oz.H_2O$, 0.02 g; chelated iron (sequestrene - (10% Fe), 10 g).

Each treatment had twenty-four plants. Pots were drenched weekly with distilled water to prevent salt accumulation. Fourteen days after plants were subjected to the deficiency treatments, when plant growth in most treatments had ceased and deficiency symptoms were apparent, four plants from each treatment group were inoculated with 0, 500, 1,000, 2,000, 5,000 and 10,000 larvae of *M. javanica*. Thirty-five days after inoculation, plants were washed free of sand. Fresh weights of tops and roots were determined and numbers of females were assessed.

Results of all experiments were analysed statistically by analysis of variance and are presented graphically as untransformed data.

Results

No stress (Fig. 1 A, B, C, D)

The number of females and fresh weight of roots increased as inoculum density increased (Fig. 1 A and 1 B). The increase in root weight with increase in infection was due to increased number and size of galls produced. Fresh weights of tops did not differ significantly (Fig. 1 C), although top growth was reduced at the lowest level of infection. Ratio of tops to roots decreased with increase in population density of nematodes (Fig. 1 D).

Effect of temperature (Fig. 2 A, B, C, D)

Soil temperature and population density had a significant effect ($P < 0.01$) on the number of females in the infected roots. The number of females in roots increased with inoculum density and temperature. Highest number of females occurred at 35°C, lowest at 15°C and intermediate at 25°C (Fig. 2 A). There was no interaction between soil temperature and inoculum levels.

Temperature had a significant effect ($P < 0.01$) on root growth (Fig. 2 B). Maximum growth occurred at 15°C and root growth was inhibited at 35°C. It also had a significant effect ($P < 0.01$) on fresh weight of tops, greatest growth occurring at 25°C and stunting was more pronounced at high soil temperature (Fig. 2 C). Although results showed a slight tendency for the tops to decrease with increase

Figure 1 (A, B, C, D)

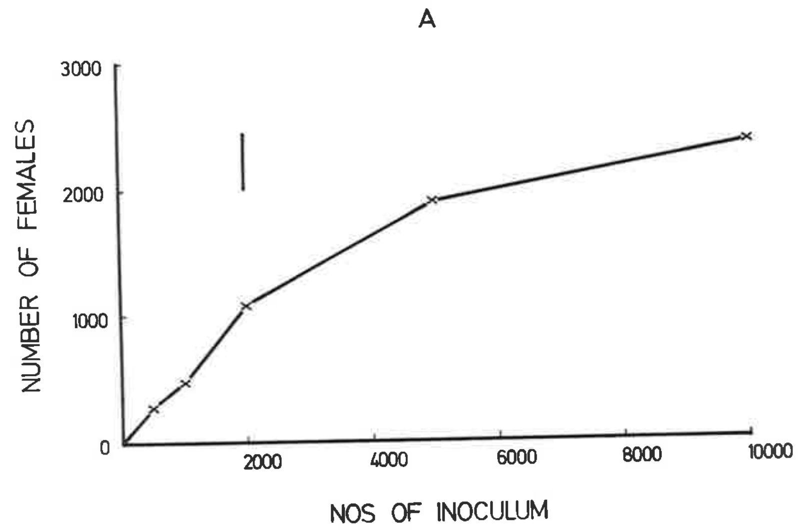
The influence of initial numbers of *M. javanica* on the fresh weight of roots and tops of tomato plants and on the number of females. Each point is the mean of four replicates. Vertical lines indicate L.S.D. at $P < 0.01$ for A, B and $P < 0.05$ for D.

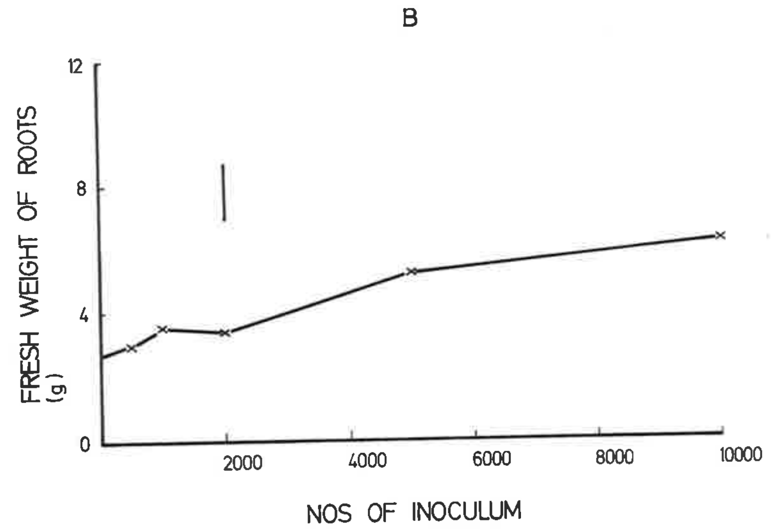
A. Number of females.

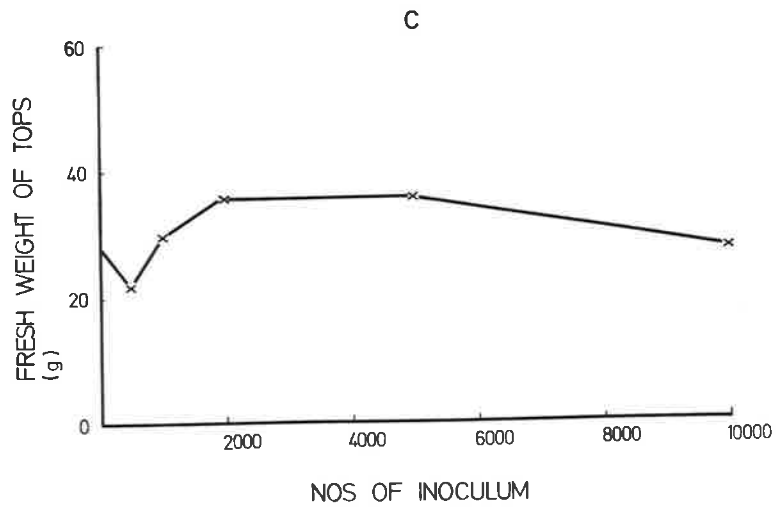
B. Fresh weight of roots.

C. Fresh weight of tops (not significant).

D. Ratio of fresh weight of tops to roots.


A
NUMBER OF FEMALES
3000
2000
1000
0
2000
4000
6000
8000
10000
NOS OF INOCULUM


B
FRESH WEIGHT OF ROOTS
(g)
12
8
4
0
2000
4000
6000
8000
10000
NOS OF INOCULUM


C
FRESH WEIGHT OF TOPS
(g)
60
40
20
0
2000
4000
6000
8000
10000
NOS OF INOCULUM

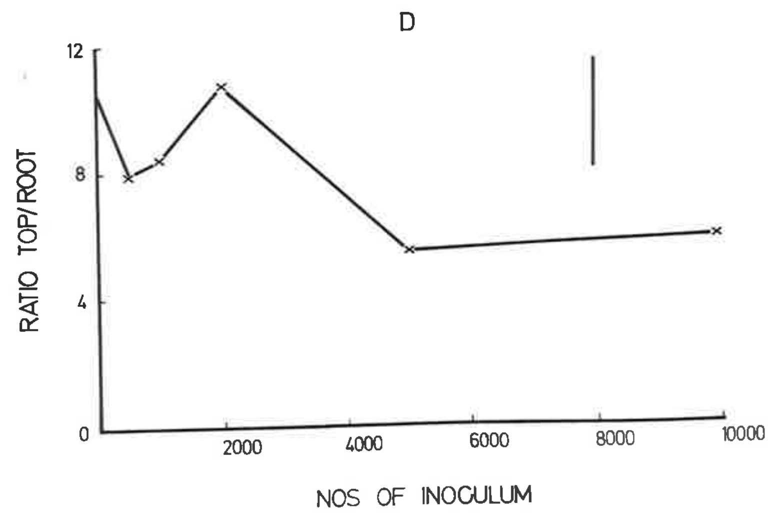

D
RATIO TOP/ROOT
12
8
4
0
2000
4000
6000
8000
10000
NOS OF INOCULUM

Figure 2 (A, B, C, D)

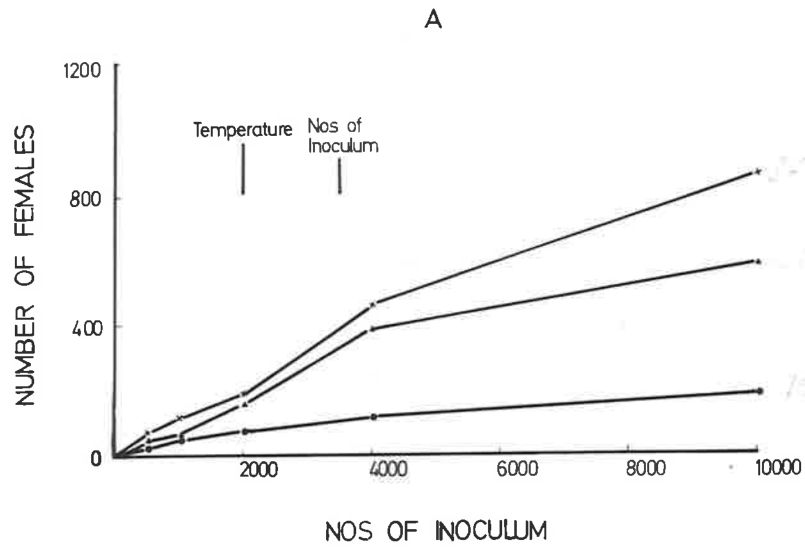
The influence of initial numbers of *M. javanica* and temperature on the fresh weights of roots and tops of tomato plants and on the number of females. Three temperatures were used: 15°C (●——●), 25°C (▲——▲), and 35°C (x——x). Each point is the mean of four replicates. Vertical lines indicate L.S.D. at $P < 0.01$.

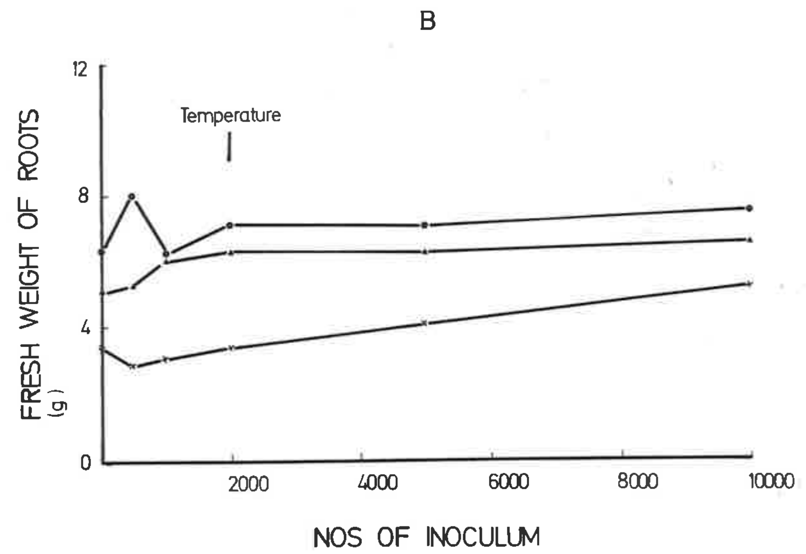
A. Number of females.

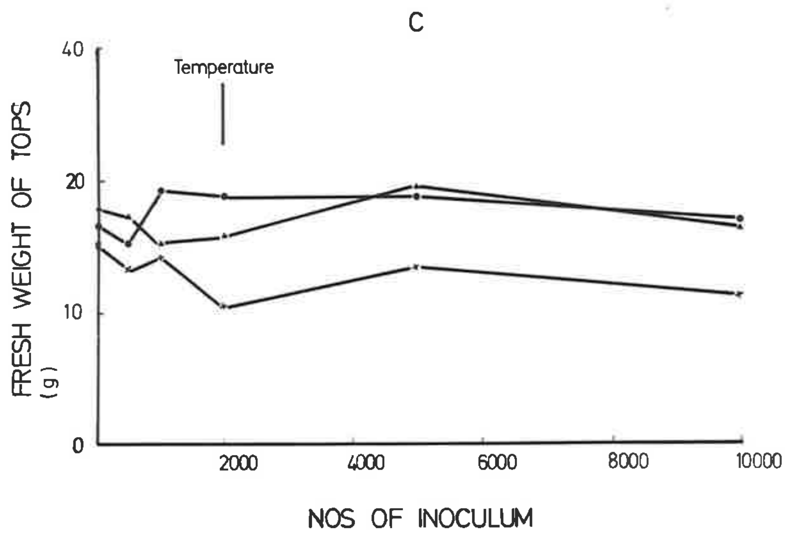
B. Fresh weight of roots.

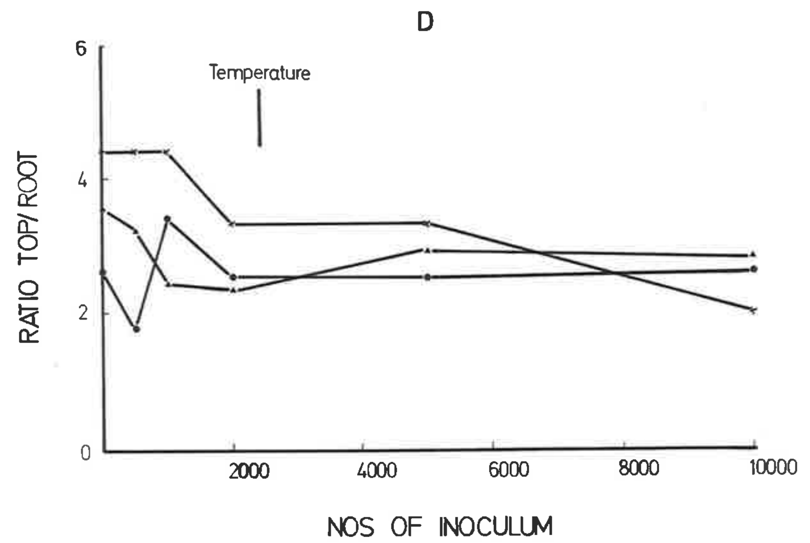
C. Fresh weight of tops.

D. Ratio of fresh weights of tops to roots.


A
1200
800
400
0
NUMBER OF FEMALES
Temperature
Nos of
Inoculum
2000
4000
6000
8000
10000
NOS OF INOCULUM


B
12
8
4
0
FRESH WEIGHT OF ROOTS
(g)
Temperature
2000
4000
6000
8000
10000
NOS OF INOCULUM


C
40
20
10
0
FRESH WEIGHT OF TOPS
(g)
Temperature
2000
4000
6000
8000
10000
NOS OF INOCULUM


D
6
4
2
0
RATIO TOP/ROOT
Temperature
2000
4000
6000
8000
10000
NOS OF INOCULUM

in population density, the difference was not significant. Galling, however, was more pronounced at higher soil temperatures. Finally, ratio of tops to roots decreased with increase in population density of nematode (Fig. 2 D).

Effect of soil moisture (Fig. 3 A, B, C, D)

Soil moisture contents and population density had a significant effect ($P < 0.01$) on number of females recovered. Number of females in the roots increased with increase in inoculum density. Highest number of females occurred at a soil moisture content of 16%, being lowest at 8% and intermediate at 25% (Fig. 3 A). There was no interaction between soil moisture and inoculum level.

Soil moisture had a significant effect ($P < 0.01$) on both fresh weight of roots and tops, the weights decreasing with decreasing moisture content (Fig. 3 B and 3 C). Inoculum levels had no significant effect on top or root growth. Ratio of tops to roots decreased with increase in inoculum levels and decrease in soil moisture content (Fig. 3 D).

Effect of nutrients (Fig. 4 A, B, C, D)

Growth of females was observed to be affected by the condition of growth medium, but no attempt was made to measure the size of the individual females. However, the number of females did not differ significantly between the nutrient levels, but was significantly different ($P < 0.01$) between inoculum density (Fig. 4 A).

Nutrients had a marked effect ($P < 0.01$) on root and top growth of tomato plants infected with *M. javanica* (Fig. 4 B and 4 C). Growth of plants was most reduced through lack of NPK, N and NK.

Figure 3 (A, B, C, D)

The influence of initial numbers of *M. javanica* and soil moisture on the fresh weights of roots and tops of tomato plants and the number of females. Three soil moisture contents were used: 25% (▲——▲), 16% (Δ——Δ), and 8% (●——●).

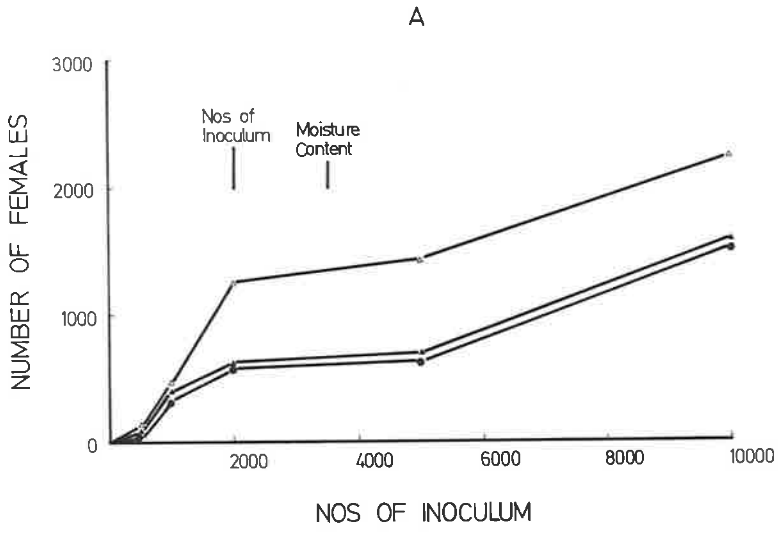
Each point is the mean of four replicates. Vertical lines indicated L.S.D. at $P < 0.01$.

A. Number of females.

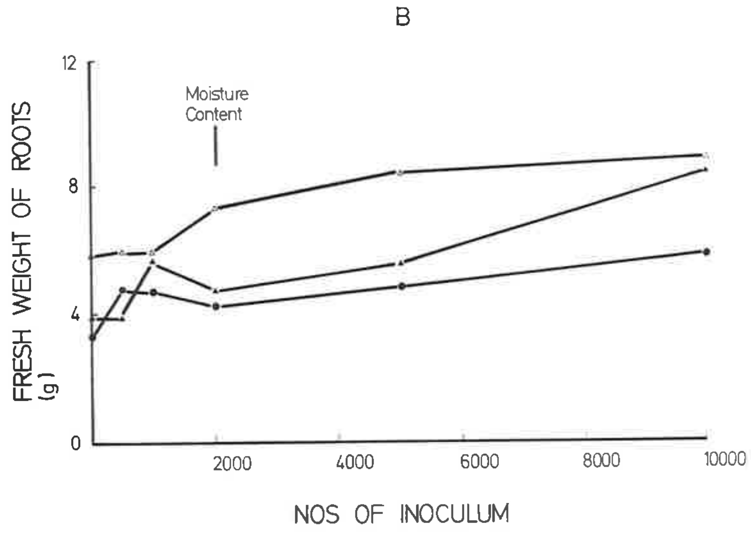
B. Fresh weight of roots.

C. Fresh weights of tops.

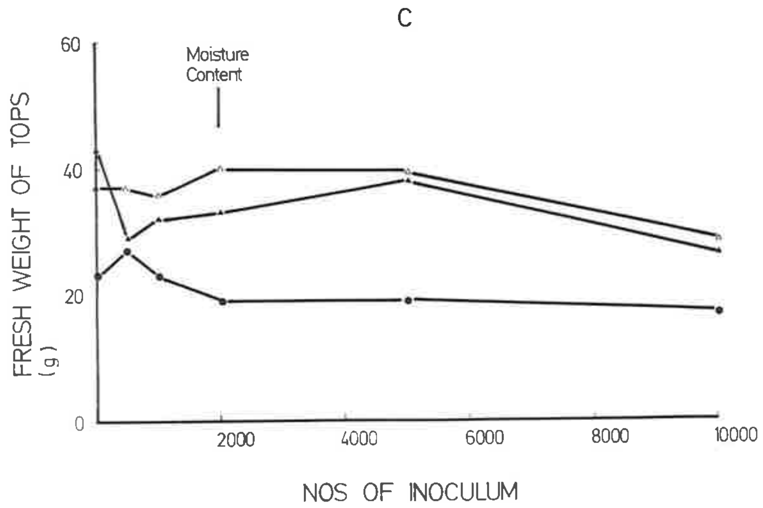
D. Ratio of fresh weight of tops to roots (L.S.D. $P < 0.05$).



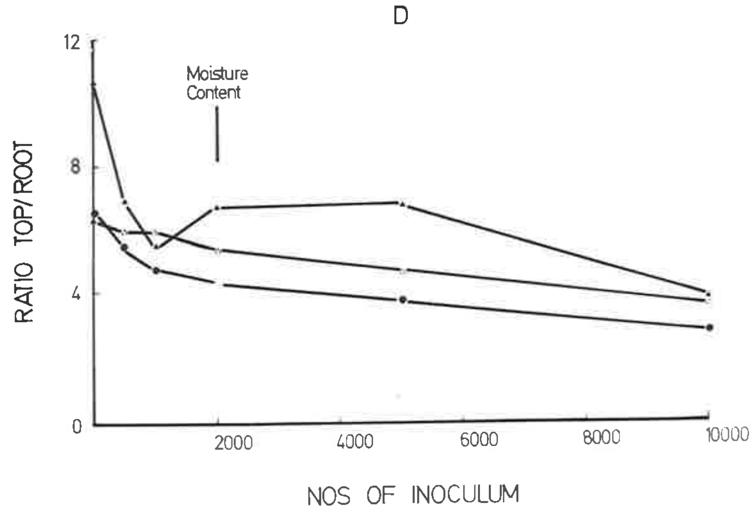
A
NUMBER OF FEMALES
3000
2000
1000
0
Nos of Inoculum
Moisture Content
2000
4000
6000
8000
10000
NOS OF INOCULUM



B
FRESH WEIGHT OF ROOTS (g)
12
8
4
0
Moisture Content
2000
4000
6000
8000
10000
NOS OF INOCULUM



C
FRESH WEIGHT OF TOPS (g)
60
40
20
0
Moisture Content
2000
4000
6000
8000
10000
NOS OF INOCULUM



D
RATIO TOP/ROOT
12
8
4
0
Moisture Content
2000
4000
6000
8000
10000
NOS OF INOCULUM

Fig. 4 (A, B, C, D)

The influence of initial numbers of *M. javanica* and soil nutrients on the fresh weights of roots and tops of tomato plants and on the number of females. Eight levels of nutrients were used: NPK (x), -NPK (⊙), -N (●), -P (o), -K (▲), -NP (Δ), -NK (■), -PK (□).

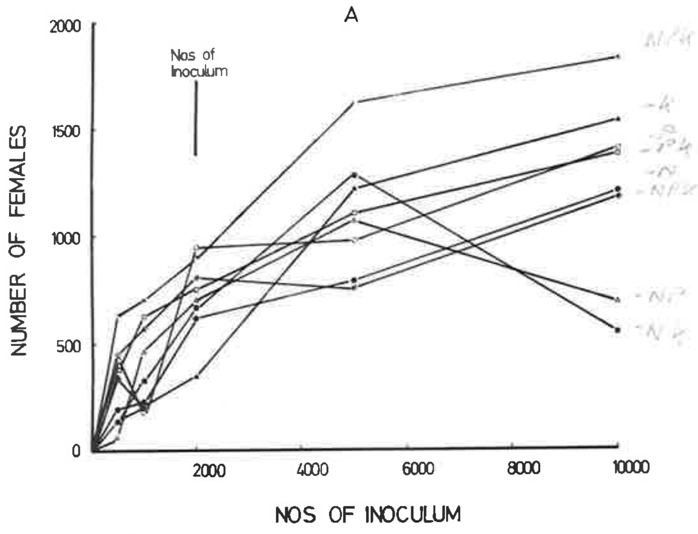
Each point is the mean of four replicates. Vertical lines indicate L.S.D. at $P < 0.01$.

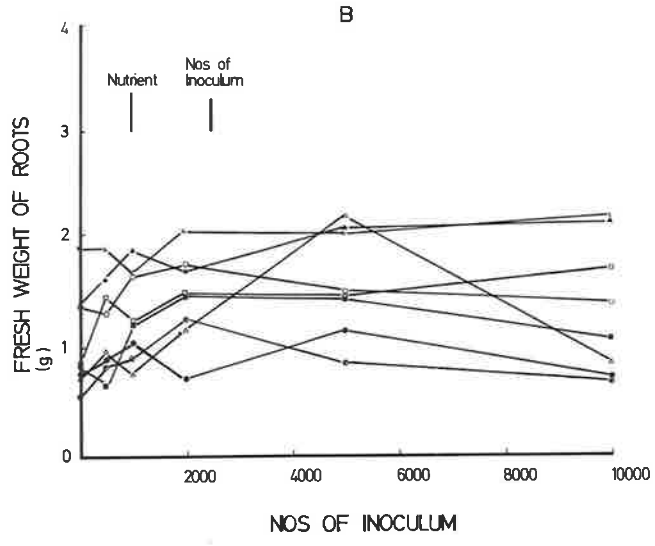
A. Number of females.

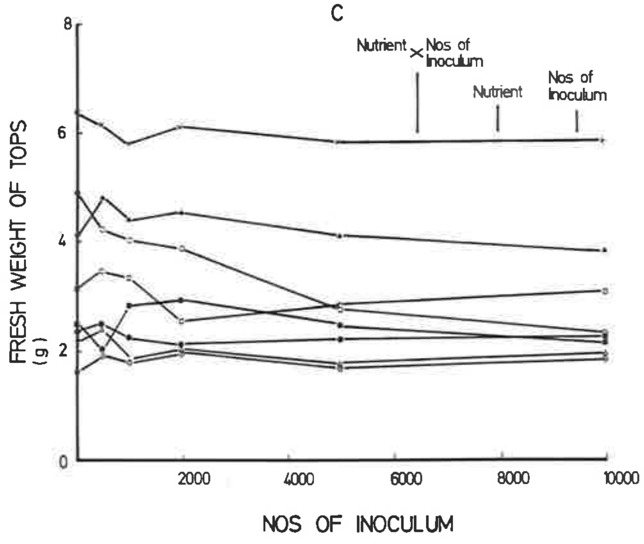
B. Fresh weights of roots.

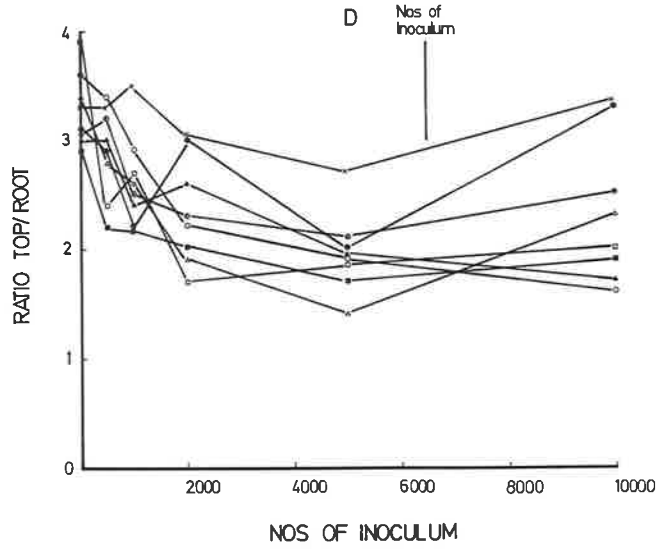
C. Fresh weight of tops.

D. Ratio of fresh weight of tops to roots.


A
NUMBER OF FEMALES
0
500
1000
1500
2000
Nos of Inoculum
2000
4000
6000
8000
10000
NOS OF INOCULUM


B
FRESH WEIGHT OF ROOTS
(g)
0
1
2
3
4
Nutrient
Nos of Inoculum
2000
4000
6000
8000
10000
NOS OF INOCULUM


C
FRESH WEIGHT OF TOPS
(g)
0
2
4
6
8
Nutrient × Nos of Inoculum
Nutrient
Nos of Inoculum
2000
4000
6000
8000
10000
NOS OF INOCULUM


D
RATIO TOP/ROOT
0
1
2
3
4
Nos of Inoculum
2000
4000
6000
8000
10000
NOS OF INOCULUM

Interactions ($P < 0.05$) occurred between the levels of soil nutrients and initial population density of nematodes.

Ratio of tops to roots decreased with increase in inoculum density merely due to increased number and size of the galls (Fig. 4 D).

5. Discussion

The data indicate that the most marked effect of *M. javanica* on the growth of tomato plants was a decrease in the ratio of fresh weight of tops to roots. With increasing inoculum density there was a gradual increase in root weight as galls became more numerous and finally there was a decrease in top weight when growth conditions were suboptimal. But in cases of optimal growth conditions, top growth did not differ significantly. Thus, top growth appeared to be maintained inspite of numerous galls supporting Seinhorst's (1963) hypothesis on tolerance level. This implies that as nematode numbers increase the decrease in top growth is low until the compensatory properties of the root system are overcome.

At low levels of nematode infection, parasites within the host's tissues stimulate the plant to utilise more of its energy in the production of more new root tissues instead of adding new top growth. This confirms earlier work by Oteifa, Barrada and Elgindi (1958). During heavy infection, most of the roots become infected with the larvae and thus the root system shows many galls. Galled tissues are capable of absorbing nutrients but plants with many galls probably cannot translocate adequate nutrients and water to the tops as efficiently as normal roots. Thus a heavy infestation leads to reduced growth. However, the effectiveness of the plant in performing this function will depend on the condition in which the plant is growing; where soil fertility is low, for example, the plant may require more roots than in a well-fertilised soil to maintain the

same top growth. Observations made on the effect of different environmental components and initial inoculum level on the growth of tomato plants support this hypothesis.

Under the experimental conditions just described some interactions between the initial population density of nematodes and some environmental factors (e.g. nutrients) on the growth of the tomato plants occurred. It seems unlikely that second order interactions involving nematodes will be apparent in multi-factorial experiments (Wallace, 1970), hence experiments, where two factors (including population density of nematodes) are being considered at a time (as described above), will probably yield just as much information on the effect of environmental factors and nematode numbers on the growth of the tomato plants. The occurrence of interactions suggests that in the field, environmental conditions may occur where the presence of nematodes causes marked reductions in plant growth. Such a situation might arise, for example, when soil conditions favour nematode infestation but inhibit plant growth.

Furthermore, the nematode density which a plant will tolerate depends on the soil conditions as well. In a well-fertilised soil, a plant may tolerate a particular population but in an infertile soil, the same population might cause marked reductions in growth and yield. But in most cases losses due to nematodes are less dramatic and whether nematodes are a cause of such losses is difficult to decide. Hence to satisfactorily assess the relative importance of nematodes in a field situation, soil fertility, soil structure, temperature, soil moisture and presence of other pathogens have to be measured as well as nematode numbers and plant growth. In relating nematode numbers to plant growth, it is the number of nematodes in the roots that should be considered; numbers in the soil are likely to be an

unreliable indication of the potential damage *M. javanica* might cause.

It should be emphasised that the hypothesis is based on data obtained from experiments using young plants; relationships between nematode numbers and top growth or yield will probably be influenced by the age of the plant (Wallace, 1970).

The hypothesis then emphasises the need for further information on the physiological responses of changes of the plant to infection by *M. javanica*. The hypothesis also raises the possibility that plants have responses that react to the activities of the nematode in the plant compensating for any damaging effects, and that a tolerance level exists. Since the concept of tolerance level is accepted, then the physiological characteristics of the plant that enable it to compensate for nematode damage can now be studied. Galls, giant cells, destruction and regeneration of new roots, formation of growth substances and inhibitors may be some of the factors that play a part in such a system.

CHAPTER IV

INFLUENCE OF *MELOIDOGYNE JAVANICA* ON THE PHYSIOLOGY OF THE HOST

Much confusion and contradiction are readily apparent when assessing the information currently available on vascular pathogenesis and its relationship to induction of symptoms. The multiplicity of symptoms expressed, and the diverse symptoms encountered from one host to another, or even in the same host under different sets of environmental conditions, all tend to hinder our efforts to unravel the complicated sequence of events linking vascular pathogenesis, host responses and the subsequent appearance of disease symptoms. Characterization of foliage wilting associated with infections by root-rotting fungi have been reported by several investigators (Deroo, 1969; Helms, Cobbs and Whitney, 1971; Powers, 1954). On the other hand, the effect of infection by root-knot nematodes on the water relations of plants has not been investigated.

In fact, the nature and extent of the injury caused by galls are not well defined. A root-knot pathogen may damage the host because it decreases root density and distribution in the soil, thereby decreasing the ability of the plant to extract water from the soil. Accordingly, reductions in the size of root systems are sometimes used to estimate the damage induced by root-knot pathogens, and there is the implication that adequate soil water can help compensate for the loss of functional roots. The symptoms associated with galling also suggest as one of the causes, some disturbance of, or interference with the sap flow in the vascular system. The giant cells usually arise from vascular cells,

so it might be expected that giant cell formation has some effect on the flow of water and nutrients through the plant roots. Thus, roots of cotton infected with *Meloidogyne incognita* are unable to transmit sufficient water to the plant to maintain normal growth under conditions of low soil moisture (O'Bannon and Reynolds, 1965). The following studies describe the histological changes caused in tomato roots by *M. javanica* and attempt to define the extent of damage associated with the foliage wilting and reduction in growth of infected plants.

1. Histological Studies

Materials and Methods

Galls of various sizes and of varying age were collected from infected tomato roots grown in experimental pots and fixed in FAA (90 ml ethyl alcohol : 5 ml glacial acetic acid : 5 ml formalin). After three days in fixative, galls were washed in running tap water and dehydrated in the tertiary butyl alcohol series. Dehydrated galls were infiltrated and embedded in paraffin. Paraffin blocks were sectioned on a sliding microtome at a thickness of 10-15μ in transverse and longitudinal planes. Sections were stained in the conventional manner using safranin and fast green (Johansen, 1940). After dehydration in an ethanol series, temporary mounts were prepared for microscopic examination.

Infected root sections (3-4 cm in length) were exposed at room temperature to pectinol 59L at pH 8 for about 5 hours, after which the roots displayed characteristic changes. The outer tissues became translucent and soft so as to be easily teased away from the vascular region. The xylem elements remained unchanged even when exposed to

the macerating enzyme for as long as 24 hours. Disintegration of the cortical tissues enabled examination of the orientation and distribution of the xylem elements in the galls.

To study the morphology of individual elements the materials were macerated in Pectinol 59L overnight, individual elements of the xylem were teased out carefully, and temporary mounts prepared for microscopic examination.

An attempt was also made to use scanning electron microscopy in histopathological studies of tomato roots infected with *M. javanica*. Galls were fixed in 3% glutaraldehyde in potassium phosphate buffer at room temperature for two to three hours. Tissues were then washed in buffer and stored overnight in the same buffer, washed in buffer and slowly dehydrated in a graded ethanol series. Tissues were embedded in paraffin, sections made, and the paraffin dissolved away with two or three changes of xylol. It was then freeze-dried and coated with gold vapour prior to loading onto the microscopic stage.

Results

Giant cells induced by *M. javanica* were observed to form in the provascular region even before the xylem pattern was established. The development of giant cells from the parenchyma cells of the vascular system inhibited cambium production, consequently no secondary xylem was formed either (Fig. 5 A). The cluster of giant cells was generally surrounded by a large number of abnormal xylem elements and the secondary thickening of the pericycle and endodermis were reduced in areas adjacent to infection sites (Fig. 5 B). The abnormal xylem was not longitudinal as were the normal elements but was disposed in a diffuse manner.

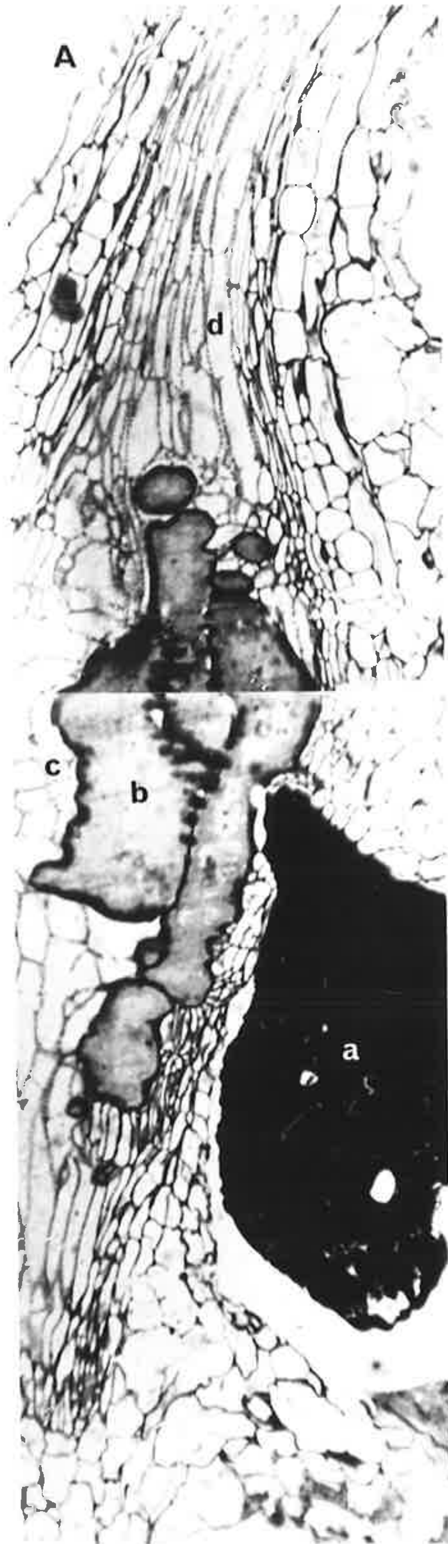
Figure 5.

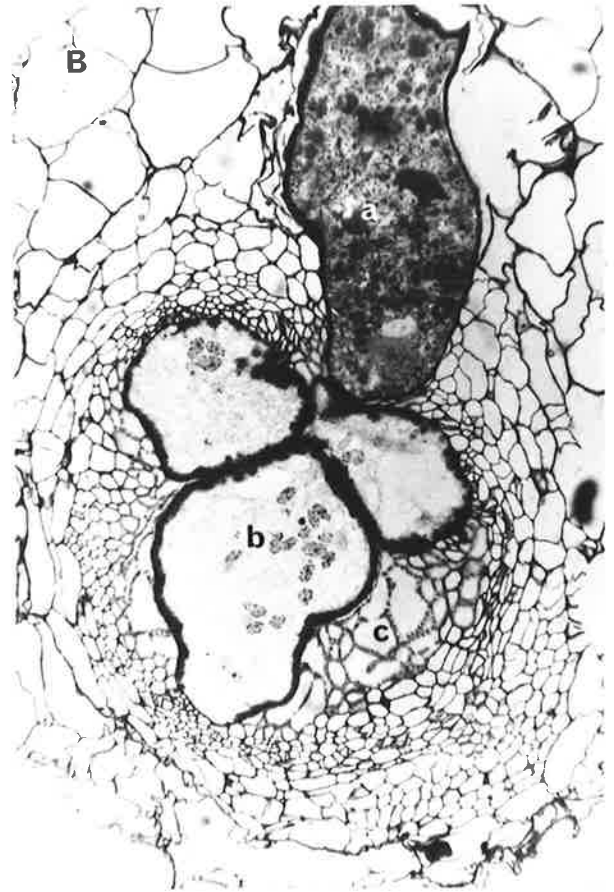
Tomato roots infected with *M. javanica*.

A. Longitudinal section showing absence of secondary xylem in the region of giant cells.

B. A cluster of giant cells surrounded by abnormal xylem.

a - nematode; b - giant cells; c - abnormal xylem vessels; d - normal xylem vessels.


A
d
c
b
a


B
a
b
c

Although vessels with wide lumina were not totally suppressed, the continuity of the larger vessels was often broken by the intrusion of giant cells into the vascular strand, thus destroying their efficiency as conducting elements (Fig. 6). Empty spaces were created in areas occupied by the giant cells and females, whereas in uninfected regions of the root, the vessels appeared normal.

Morphology of the abnormal xylem differed from that of the normal ones. The fragments of xylem separated out possessed no definite shape or sizes, some were perforated, while others were not (Fig. 7). Normal vessels were usually longer and thinner.

Scanning electron microscope studies of the galls showed a similar pattern (Fig. 8 A and B). Sections showed discontinuity of xylem vessels and clusters of abnormal xylem produced around the giant cells.

2. Measurement of Root Resistance by 'Bomb Calorimeter'

Materials and Methods

Histopathological studies of *M. javanica* infected tomato roots suggest that the absence of secondary xylem and discontinuity of xylem vessels provides a natural barrier to efficient absorption and translocation of water and nutrients up the plants. To further test this notion, the degree of interference imposed through galling was assessed in infected and noninfected tomato and between various levels of initial inoculum density.

Single tomato seedlings were grown in 10 cm plastic pots in John Innes potting compost for forty days, after which larvae of

Figure 6

Photograph showing the discontinuity of the xylem vessels in the region of infection (low power).

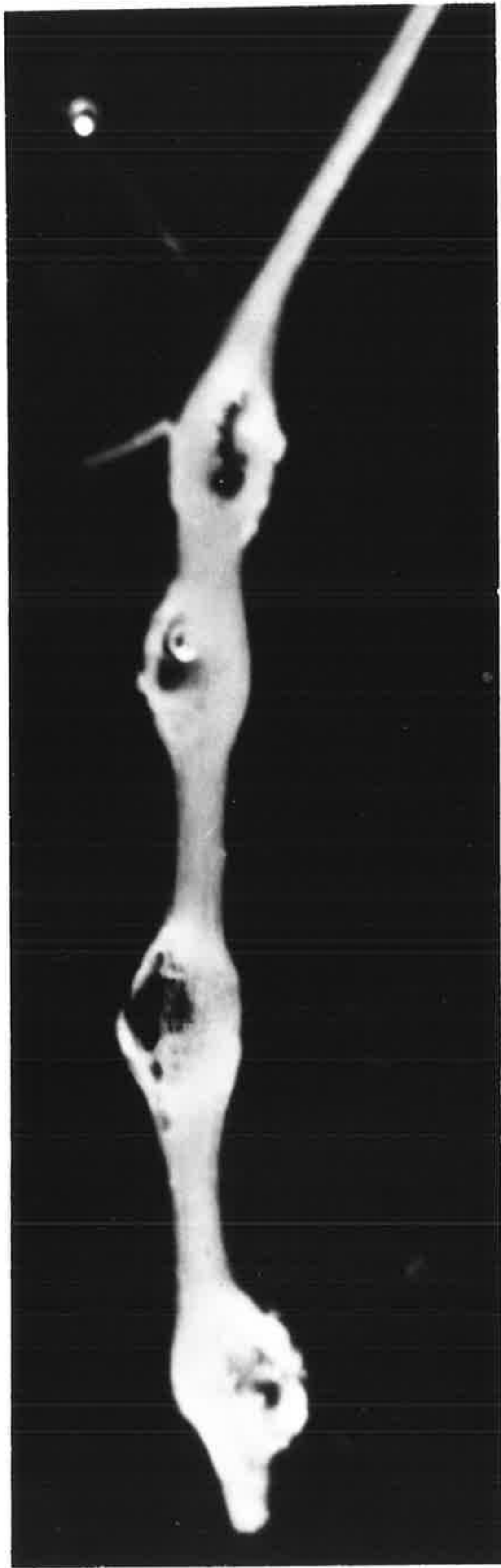


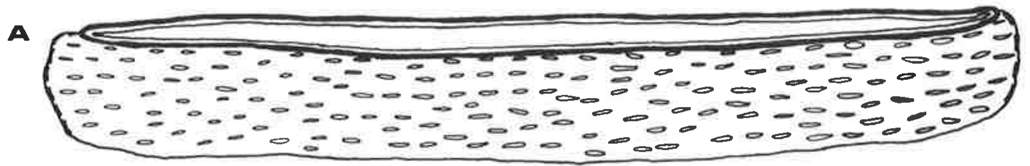
Figure 7

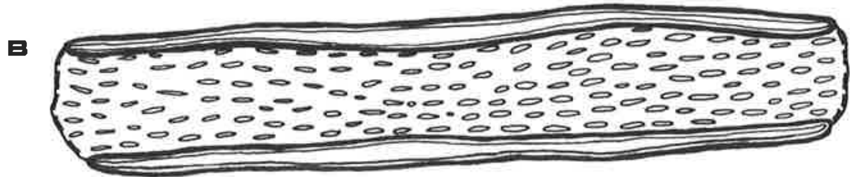
Diagram showing macerated tracheal elements of tomato roots infected with *M. javanica*.

A and B: Vessel elements of normal secondary xylem.

C - H: Vessel elements of abnormal xylem with perforations.

I and J: Non-perforated elements of abnormal xylem.


A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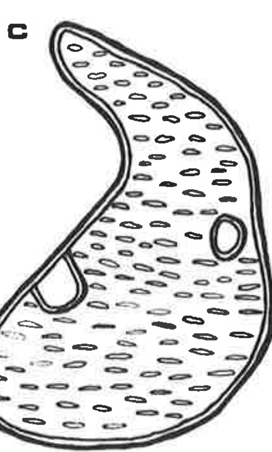
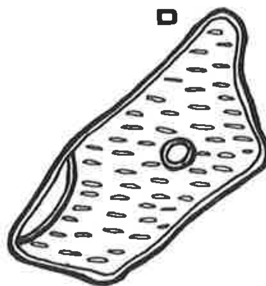
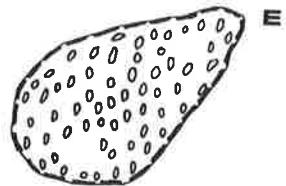
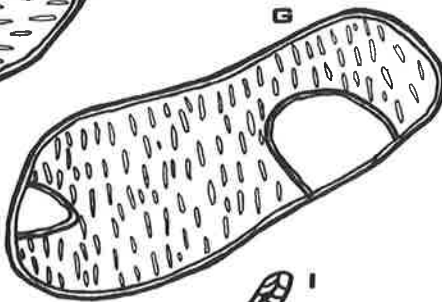
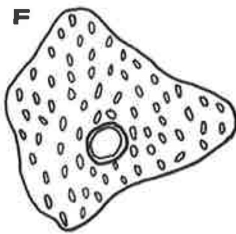

C


D


E


G


F

J

I

H

0 140μ

Figure 8 A.

Scanning electron microscopy of tomato roots infected with *M. javanica*.

Longitudinal section showing absence of secondary xylem vessels.

a - nematode; b - abnormal xylem vessels;
c - normal xylem vessels.

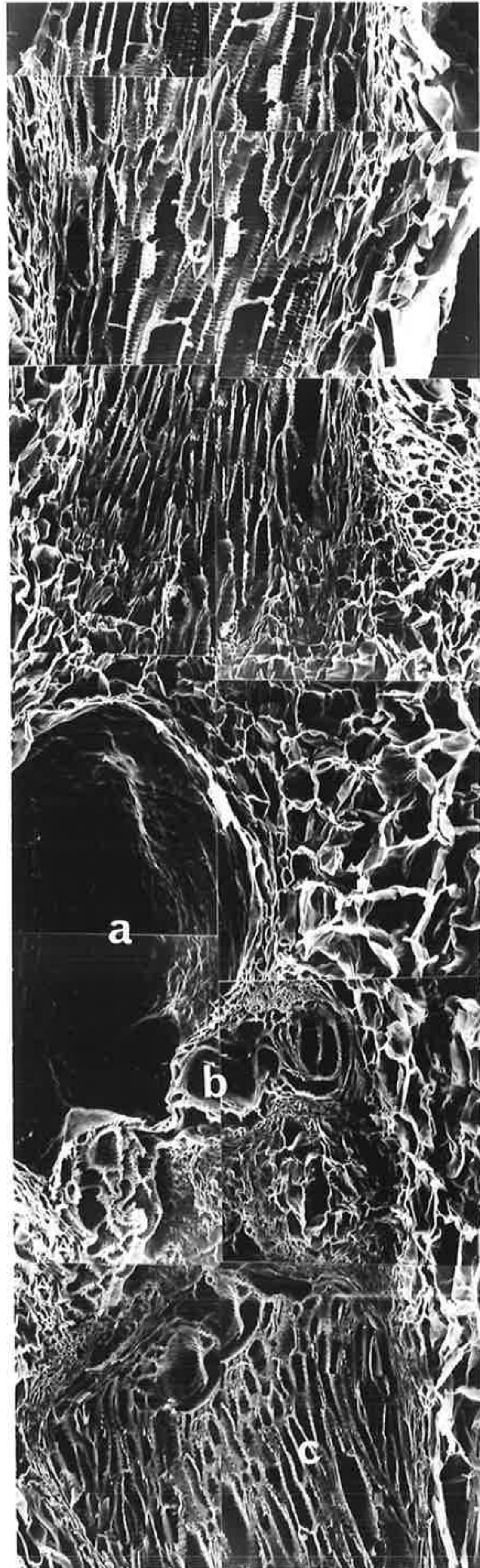
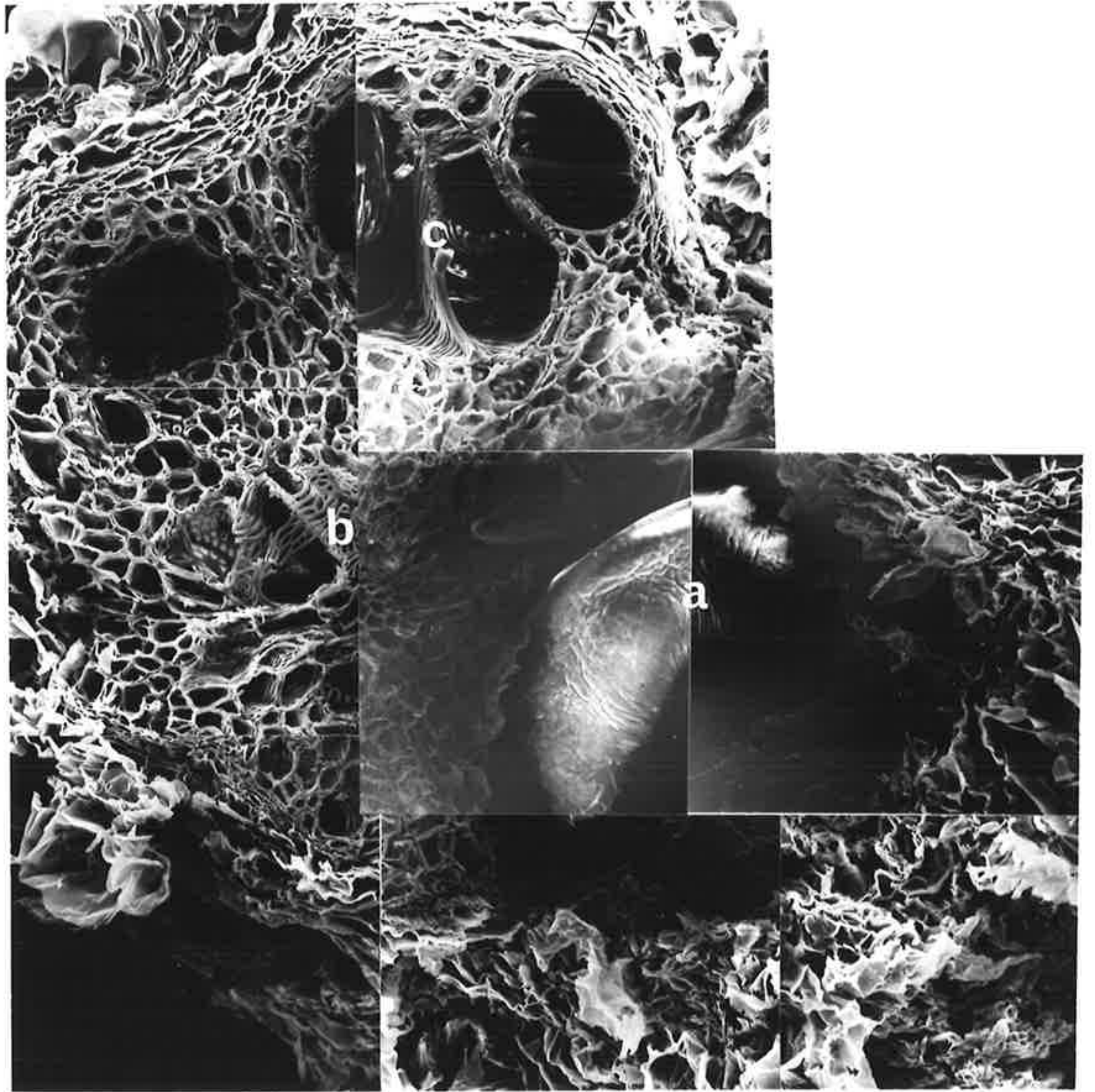

a
b
c

Figure 8 B.

Scanning electron microscopy of tomato roots infected with *M. javanica*.

Cross-section showing presence of abnormal xylem around the infection site.

a - nematode; b - abnormal xylem vessels; c - normal xylem vessels.


c
b
a

M. javanica were inoculated into the soil at the base of each plant. Inoculum densities used were 0, 500, 1,000, 2,000, 5,000 and 10,000. The plants were then subjected to three moisture contents of high (25%), medium (16%) and low (8%), three days after inoculation to ensure maximum penetration. Moisture in each pot was kept constant by weighing to constant weight daily. The pots were randomly arranged on the glasshouse benches with fluctuating temperatures of 20°C-30°C. Thirty-five days after inoculation, the plants were cut off approximately four cm from the soil level and the degree of interference due to galling (root resistance) was measured by using the modified apparatus of Melhus *et al.* (1924), designated as 'Bomb Calorimeter'. The amount of pressure required to drive the first few drops of water to the cut surface of the stem was taken to be directly proportional to the resistance of the root system.

3. Mineral Concentration and Distribution

At the end of the experiment, the tops and roots were weighed, oven-dried, and again weighed. The dried tops and roots were finely ground in the vial of a M.V.T.100 mill. Pellets weighing 2-3 g were pressed from each sample under a load of 6,804 kg delivered by an hydraulic press with 6.51 cm diameter ram. Concentration of the various elements (K, P, Mg, Cl, Fe, S) were determined using a Phillips PW 1540 x-ray fluorescence spectrophotometer.

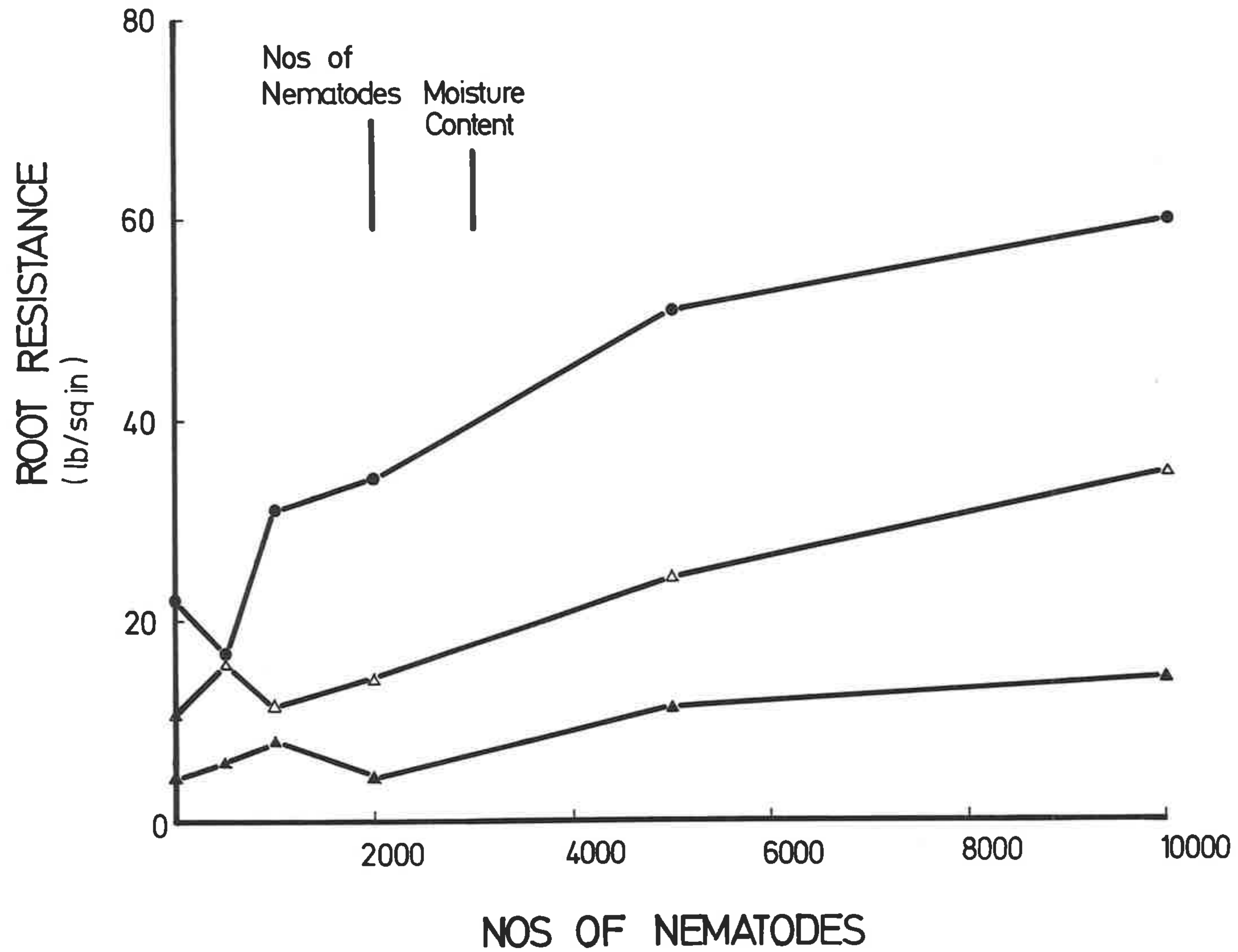
Statistical analysis was carried out on the results which were expressed graphically as untransformed data.

Results

Population density and soil moisture had a significant effect ($P < 0.01$) on root resistance. Resistance increased with decrease in soil moisture content and increase in nematode population

Figure 9.

The influence of *M. javanica* on the root resistance of tomato plants at various soil moisture contents, forty days after inoculation. Soil moisture contents used were - 8% (●), 16% (Δ) and 25% (▲) w/w. Each point is the mean of four replicates. Vertical lines indicate L.S.D. at $P < 0.01$.



80
60
40
20
0
ROOT RESISTANCE
(lb/sq in)
Nos of
Nematodes
Moisture
Content
2000
4000
6000
8000
10000
NOS OF NEMATODES

(Fig. 9). Highest root resistance was obtained at highest initial population density coupled with low soil moisture. It is not clear in this case how the observed effects should be apportioned between nematode infection and soil moisture.

To eliminate the possibility of soil moisture having an effect on root resistance, a similar experiment was carried out where plants were inoculated with 5,000 larvae of *M. javanica* and subjected to two moisture extremes (low and high). Resistance was measured as above, after which all the pots were flooded and the root resistance measured every two hours for a period of 24 hours.

The resistance was initially high at low moisture level but after flooding, resistance decreased in both cases similarly (Fig. 10). The decrease in root resistance was greater in the case of low moisture treatment, on the other hand the high moisture treatment assumed a fairly constant decline.

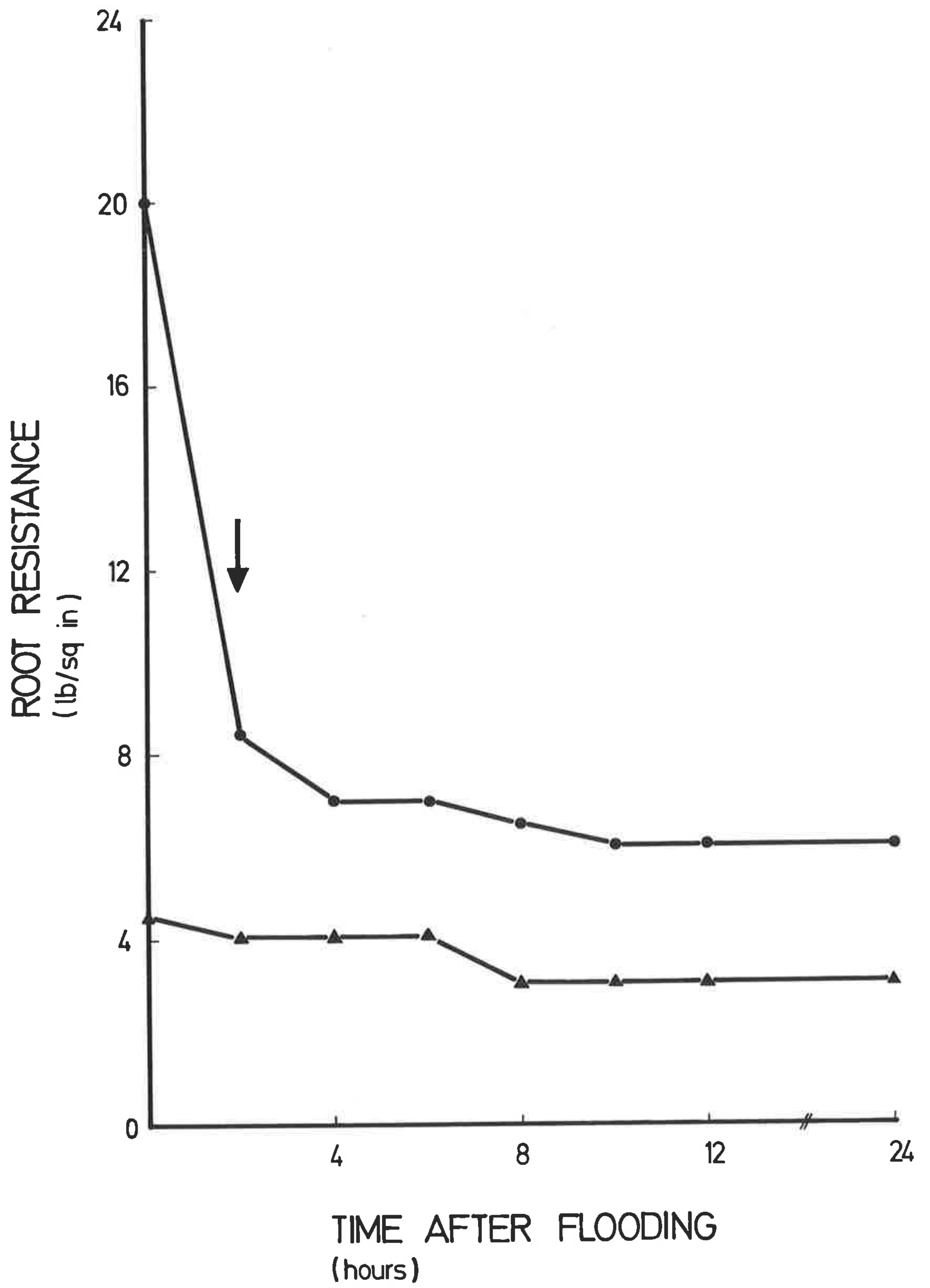
The effect of nematodes on the nutrient status of tomato was measured on total accumulation and percentage concentration of potassium, phosphorus, magnesium, chlorine, sulphur and calcium. Concentration in the leaves of the infected plants did not show consistent or serious deviations (Table I). Heavily galled root systems showed the tendency to have increased mineral content. Even though concentration of some elements were below those of the control, the actual values were well within limits needed for normal growth. Owing to the short experimental period, sensitivity of tomatoes to root-knot infection cannot be precisely determined.

4. Water Relations

Disruption and abnormality of xylem occurred when giant cells were formed in response to infection by the root-knot nematode. This

Figure 10.

The influence of flooding on root resistance of tomato plants, forty days after inoculation with *M. javanica*. Soil moistures used were 8% (●) and 25% (▲) (w/w). Arrow indicates initiation of flooding.



24
20
16
12
8
4
0
ROOT RESISTANCE
(lb/sq in)
4
8
12
24
TIME AFTER FLOODING
(hours)

Table I

Percentage distribution of potassium, phosphorus, chlorine, magnesium, sulphur and calcium in tops and roots of tomato plants infected with *M. javanica*. Each value is the mean of four replicates.

TOPS

No. of Nematodes	Phosphorus	Potassium	Magnesium	Chlorine	Sulphur	Calcium
0	0.44	2.43	0.82	0.69	0.44	1.69
500	0.51	2.58	0.69	1.49	0.63	1.23
1000	0.48	2.58	0.66	1.24	0.56	1.09
2000	0.43	2.39	0.62	1.01	0.54	1.12
5000	0.37	2.50	0.59	0.88	0.46	1.25
10000	0.41	2.57	0.65	0.92	0.45	1.38

ROOTS

No of Nematodes	Phosphorus	Potassium	Magnesium	Chlorine	Sulphur	Calcium
0	0.45	2.30	0.90	1.03	0.39	0.74
500	0.42	2.69	0.56	1.38	0.29	0.64
1000	0.44	2.12	0.70	1.05	0.32	0.70
2000	0.46	2.48	0.67	1.15	0.31	0.69
5000	0.51	2.54	0.69	0.96	0.35	0.67
10000	0.51	2.37	0.70	0.87	0.39	0.69

causes an increased resistance to absorption and sap flow leading to an inducement of internal water stress in the infected plants. All plant physiological processes depend on water and if growth and development are to proceed normally internal water stress must not develop within the tissues (Gates, 1972). Moisture stress inhibits such processes as photosynthesis (Boyer, 1970), transport to the shoot system of cytokinins synthesized in root tips (Itai and Vaàdia, 1971) and disruption of organelles and other cell components (Todd, 1972).

Thus, the purpose of the following experiments was to determine the effect of *M. javanica* on the water relations of the host. Measurements of leaf water-potential and water vapour loss from leaves and rate of transpiration were selected as an accurate means of estimating the influence of root-knot on the internal water status of healthy and nematode-infected plants.

Materials and Methods

(a) Diffusive Resistance

Single tomato seedlings were grown in 10 cm pots in John Innes potting compost for forty days, after which they were inoculated with 6,000 larvae of *M. javanica*. Uninoculated plants were used as controls. Pots were arranged randomly in the growth cabinet with temperatures of 20°C-25°C and 12 hours of light. Relative humidity and light intensity were kept constant throughout the experimental period. Measurements commenced one week after inoculation and thereafter were made at intervals of seven days for a period of eight weeks to allow maximum damage to be done to the root systems. Soil was brought to field capacity at the beginning of each measurement. Diffusive resistance

of abaxial leaf surfaces of healthy and infected tomato plants was measured with an aspirated diffusive porometer (Byrne, Rose and Slatyer, 1970). Measurements were carried out 12 hours after saturation of the rooting medium with water in the light period. Raw data in seconds, collected from diffusive resistance measurements of the third pair of true leaves at similar physiological ages were converted to sec.cm^{-1}. The same leaves were then detached and immediately placed into a thermocouple for determination of water potential.

(b) Leaf water potential

A peltier-cooled thermocouple psychrometer was used to measure leaf water potential (Barrs, 1968). The leaves were wrapped around a wire mesh insert protecting the thermocouple and pushed into the psychrometer chamber, which was then stoppered. The chamber was then placed in a water bath (25°C $\pm$ 0.01°C) and allowed to equilibrate for at least two hours before reading the thermocouple output. The water potential was calculated by comparing the recorded deflections with the deflections obtained from a graded series of sodium chloride solutions.

(c) Transpiration

Transpiration rate was studied by a gravimetric analysis involving growing plants in 500 ml waxed paper cups containing sand. The plants were inoculated with 1,000 larvae of *M. javanica*. Uninoculated plants were used as controls. A closed system surrounding the roots was provided as follows: a drain hole was made in the bottom of the cup, and a watering tube inserted into the sand. The surface

was then covered with polystyrene beads. Each cup was inserted into a second cup and the watering tube sealed with modelling clay. At the beginning of each measurement, the outside cup was removed and Hoagland's solution added through the watering tube until the sand was saturated. The system was allowed to drain for 30 mins. and resealed. The difference in weight of the saturated system after 24 hours was expressed as the rate of transpiration in g/24 hours.

The results were analysed statistically by regression and are represented graphically as untransformed data.

Results

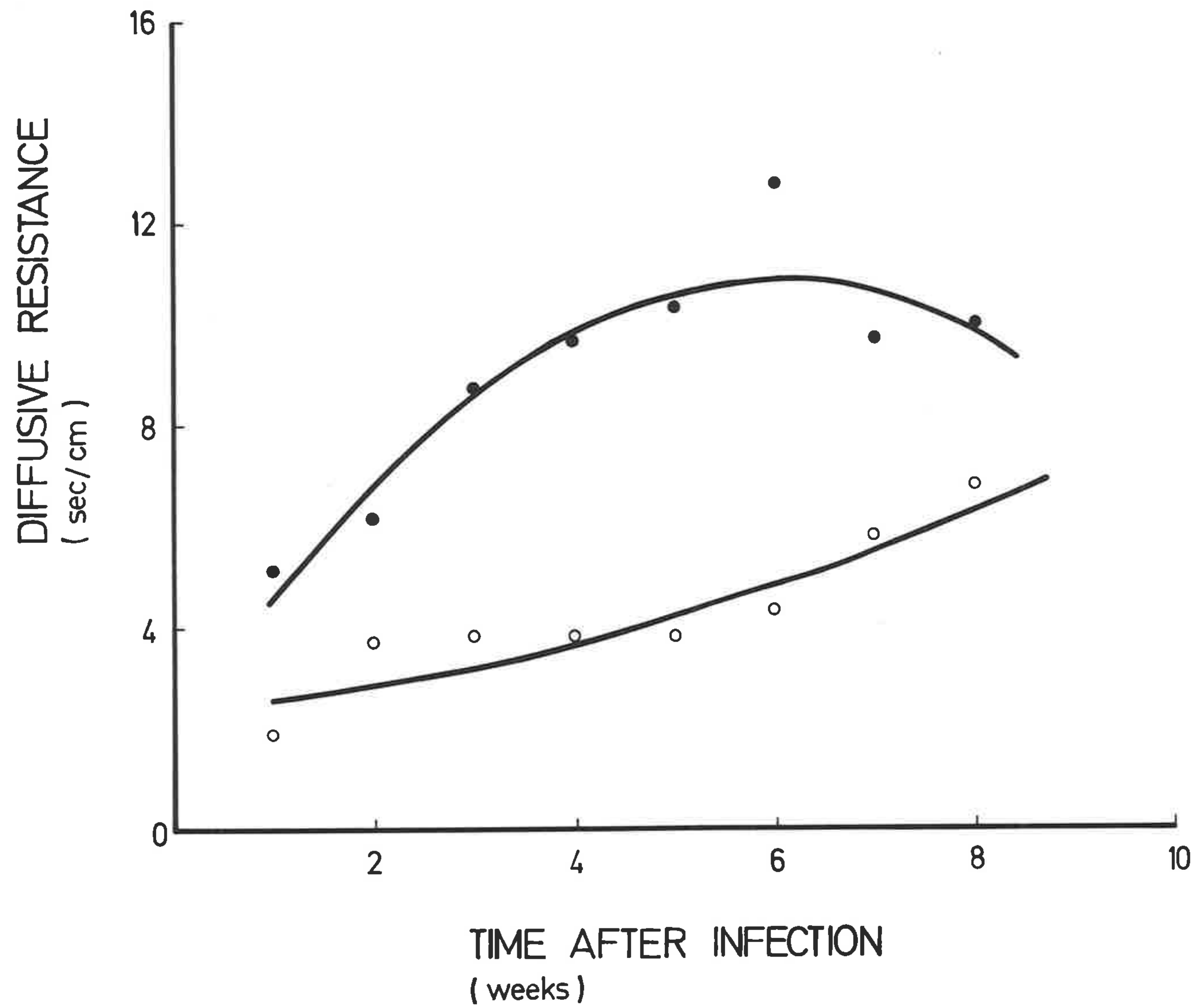
The diffusive resistance of tomato plants infected with *M. javanica* increased as infection progressed and was always higher than in uninfected plants (Fig. 11). Regression coefficients were significantly different ($P < 0.05$) between the infected and non-infected plants. The resistances of leaves obtained from non-infected plants were lower than those from infected plants at any given time after inoculation. The infected plants did not show any significant decline in growth at the end of the experiment.

Water potential of leaves from infected and healthy plants over an extended period of 8 weeks after inoculation is shown in Fig. 12. The regression coefficients were significantly different ($P < 0.05$). Water potential in the infected plants decreased with time after inoculation at a greater rate than that of healthy plants. Inoculation of the tomato plants with 6,000 larvae of *M. javanica* did not produce stunting or other foliar symptoms.

Figure 11.

Regression lines showing the relationship between time and diffusive resistance of tomato plants with (●) and without (o) *M. javanica*.

Each point is the mean of six replicates.



16
12
8
4
0
DIFFUSIVE RESISTANCE
(sec/cm)
2
4
6
8
10
TIME AFTER INFECTION
(weeks)

Figure 12.

Regression lines showing the relationship between time and water potential of tomato plants with (●) and without (o) *M. javanica*.

Each point is the mean of six replicates.

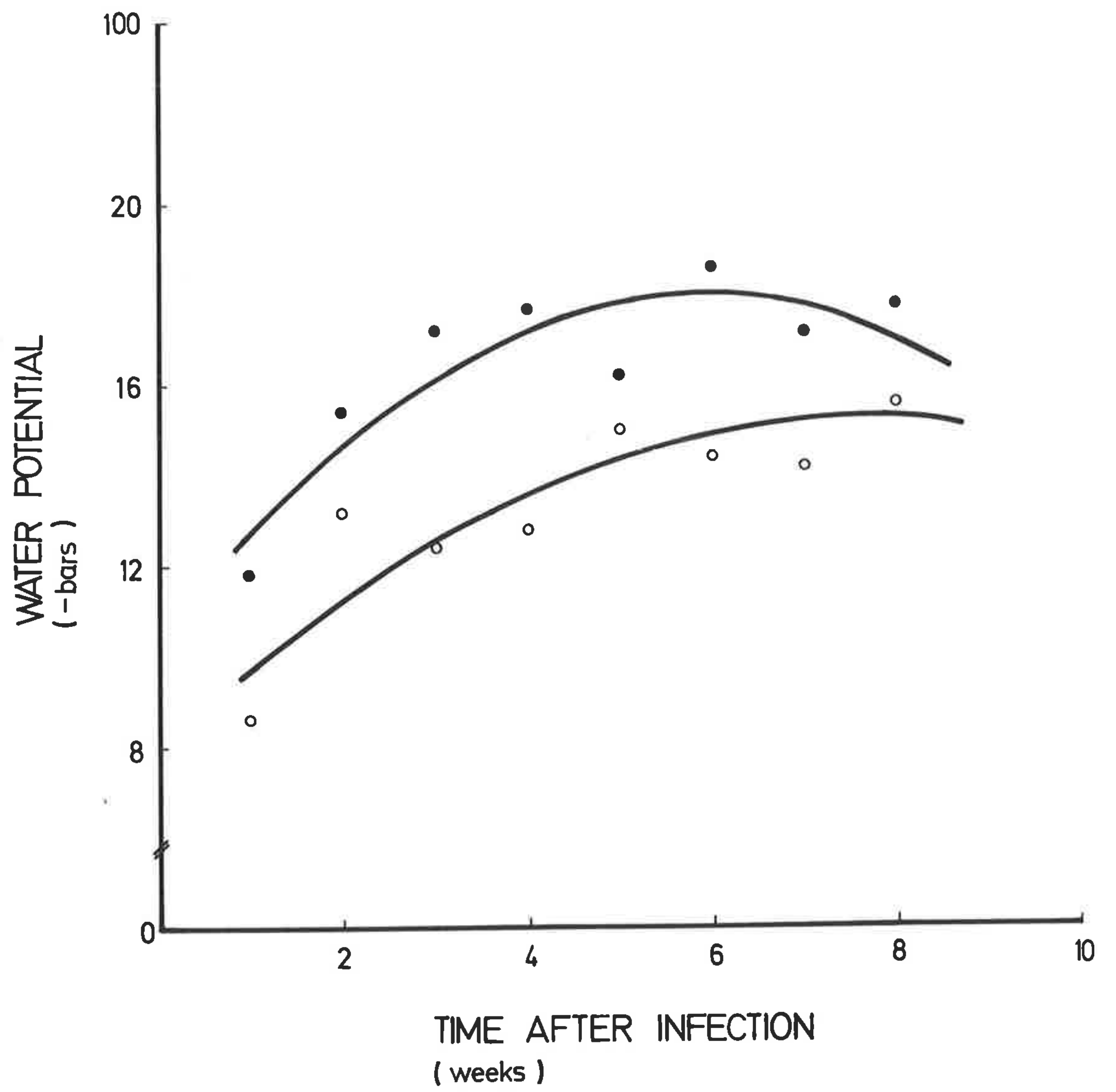

100
20
16
12
8
0
WATER POTENTIAL
(-bars)
2
4
6
8
10
TIME AFTER INFECTION
(weeks)

Figure 13.

Regression lines showing the relationship between water potential and diffusive resistance of tomato plants with (●) and without (o) *M. javanica*.

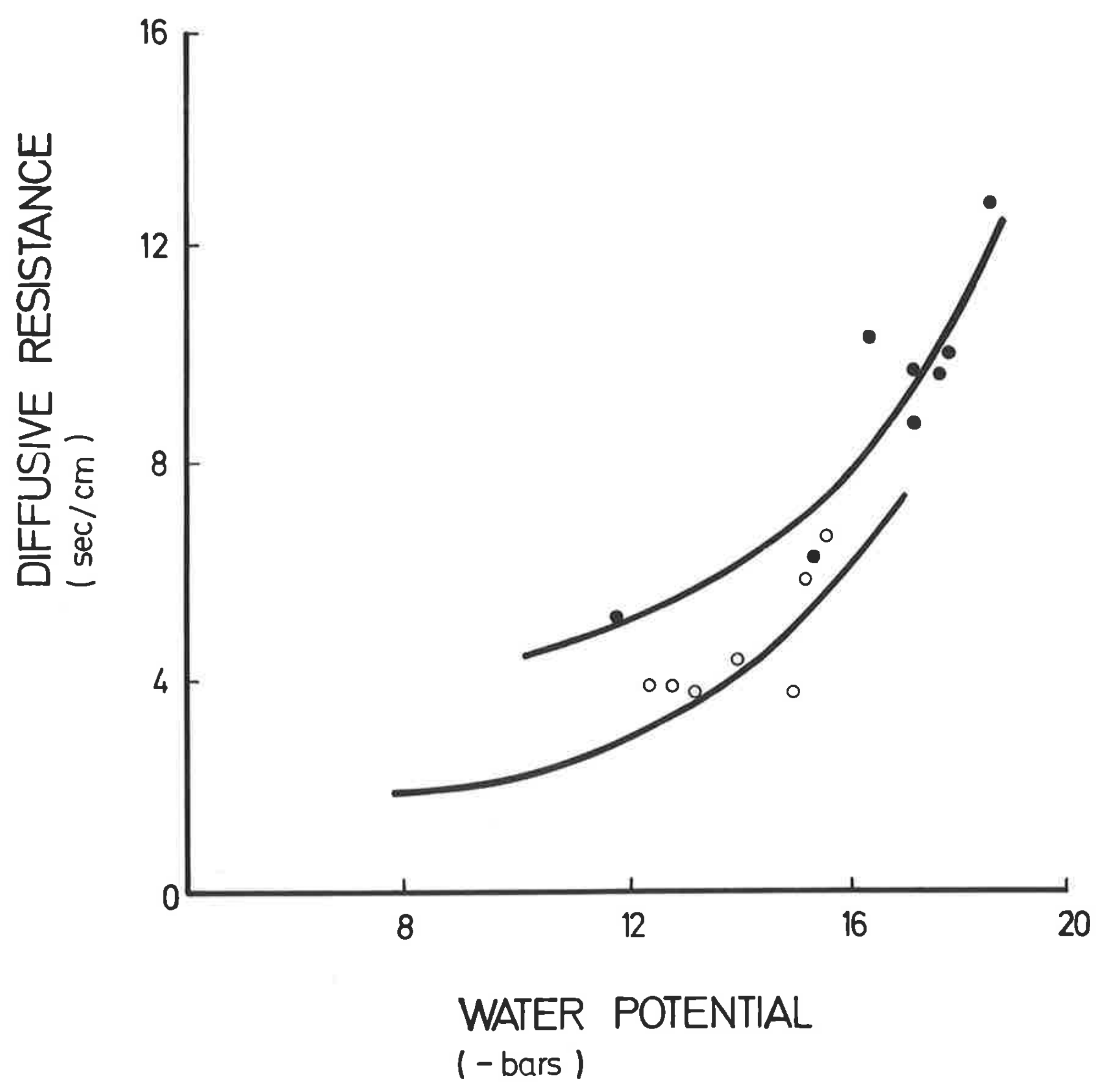

DIFFUSIVE RESISTANCE
(sec/cm)
0
4
8
12
16
WATER POTENTIAL
(- bars)
8
12
16
20

Fig. 13 shows the relationship between leaf diffusive resistance and water potential for both the infected and healthy plants. The leaf diffusive resistances were essentially unaffected by a drop in water potential to about -12 bars. Below 12 bars the diffusive resistances increased sharply. Even though the data are somewhat scattered, the regression lines drawn indicate that the diffusive resistance of leaves following inoculation was invariably higher ($P < 0.05$) than the diffusive resistance of healthy plants at the same water potential values.

Transpiration rates of whole infected tomato plants over a period of eight weeks after inoculation did not differ significantly (Fig. 14) from those of the uninfected healthy plants. The plants did not show any significant visual stunting, wilting or other foliar symptoms throughout the experimental period.

5. Hormonal imbalance

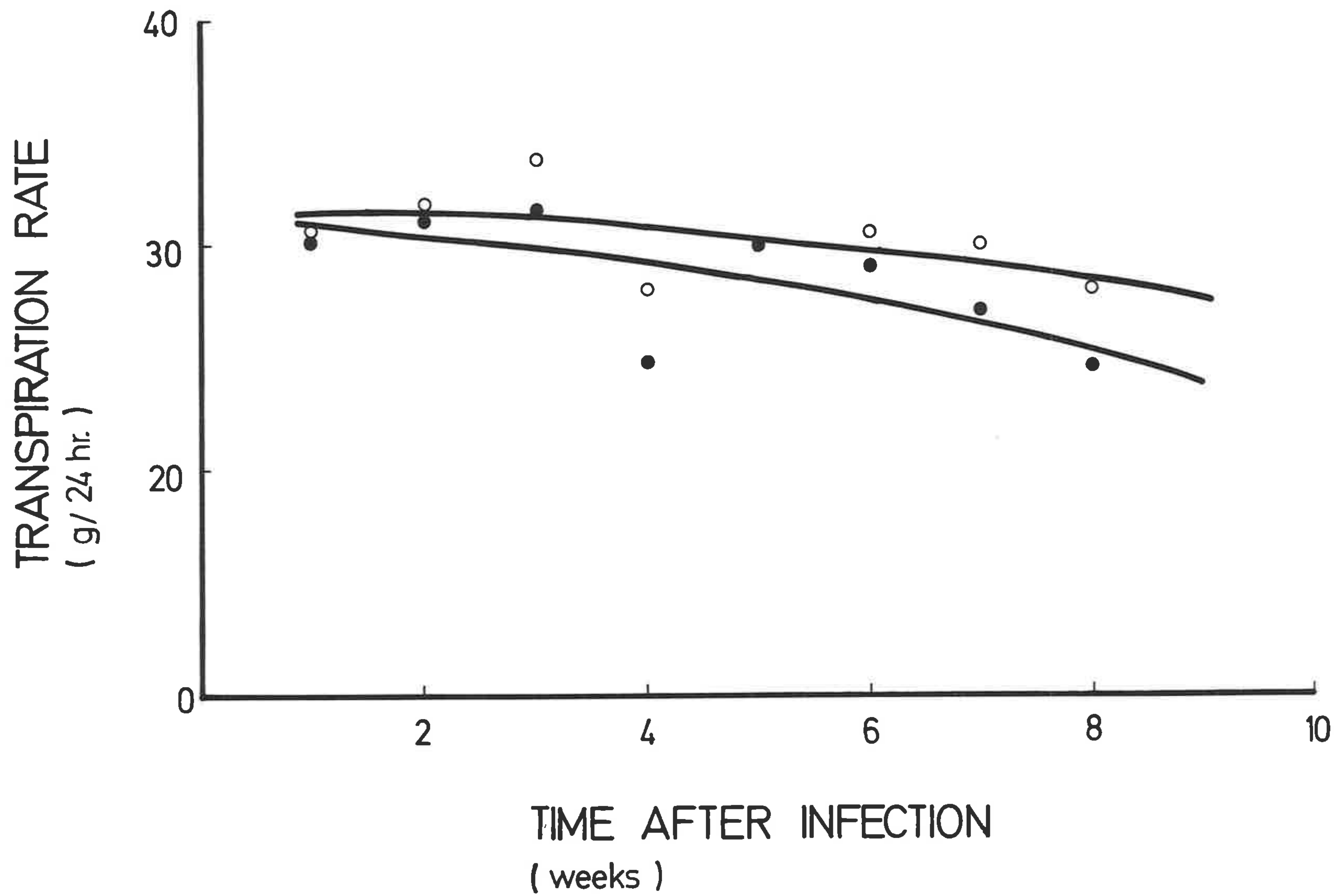
Since the observed growth reduction and wilting could not be totally associated with lack of water and nutrients, the possibility of an imbalance of growth regulators should be considered. Neoplastic growth at the infection site has encouraged speculation that growth regulators are involved. Small induced increases in growth regulators by sub-injurious root-knot infection could be a possible explanation for increased growth, whereas growth reduction at higher infection levels could result from growth regulators being increased to toxic concentrations.

The interpretation of the results of the experiments above does not preclude the possibility that infected plants in the field do not suffer from water and nutrient deficiency. Instead, the greatly reduced surface area of roots of infected plants would be a distinct disadvantage when nutrients and water supplies were marginal. But

Figure 14.

Regression lines showing the relationship between time and rate of transpiration of tomato plants with (●) and without (o) *M. javanica*.

Each point is the mean of six replicates.



TRANSPIRATION RATE
(g/ 24 hr.)
40
30
20
0
2
4
6
8
10
TIME AFTER INFECTION
(weeks)

these experiments do show that root-knot infection can also cause growth reduction when the plant has sufficient water and nutrients. Therefore, an attempt was made to study the changes in gibberellins, cytokinins, and abscissic acid concentration in roots and tops of healthy and infected tomato plants.

Materials and Methods

(a) Gibberellins

Tomato seeds were germinated and grown in vermiculite for 28 days, after which they were transplanted to John Innes potting compost in 12 cm pots. Ten plants of uniform size were inoculated with 7,000 larvae of *M. javanica*, and uninoculated plants were used as controls. The plants were arranged randomly on the glasshouse benches with temperatures fluctuating between $20^{o}C$-$30^{o}C$. At nine weeks of age, the plants were cut off at the cotyledonary node, and the xylem exudate collected over a period of 48 hours. The exudate collected was divided into two portions and lyophilised. Root tissues from the uninfected and galls from the infected plants were collected and lyophilised too.

The gibberellins in the exudate were extracted using the modified procedure of Goldschmidt and Monselise (1968). The filtrate was concentrated under vacuo, adjusted to pH 8 with 3N NH_4OH and extracted twice with ether. The ether fractions were collected, and the residue was pooled and acidified to pH 3. It was extracted twice with ethyl acetate and again with n-butanol.

A 1-gm sample of lyophilised root tissues was homogenised in 80% ethanol and refrigerated overnight. Following filtration, the filtrate was concentrated under vacuo at $40^{o}C$ and extracted with ether.

All ether extracts were pooled, neutralised and evaporated to dryness under vacuo. The residue was redissolved in 2 ml ethanol. Each fraction was strip-loaded onto previously acid-washed Whatman No. 1 paper and chromatographed descending in isopropanol : NH_4OH : water (10 : 1 : 1 v/v). The air-dried chromatograms were sectioned and gibberellin activity was assayed by the barley endosperm test (Coombe, Cohn and Paleg, 1967a and b). Barley used in the assay was the cultivar 'Clipper' and the assay was run in duplicate.

(b) Cytokinins

The residue obtained from the second portion of lyophilised exudate and tissues was used for determination of cytokinin activity. The filtrate obtained was concentrated under vacuo at 40°C and adjusted to pH 7 with barium hydroxide. After extracting twice with water-saturated butanol, the butanol fractions were pooled and evaporated to dryness. The residue was redissolved in 80% ethanol and appropriate quantities were transferred to assay flasks. Cytokinin activity was assayed using the soybean callus test (Miller, 1963). The assay was run in triplicate. The fresh weight of the callus was determined four weeks after culture. The increase in fresh weight of callus is directly proportional to the concentration of cytokinins present in the extract.

(c) Abscissic Acid

For determination of abscissic acid, 20 preweighed one cm sections randomly taken from tops or roots were homogenised in an acetone : water mixture (1 : 1 v/v), adjusted to pH 9 with 3N NH_4OH and centrifuged. The supernatant layer was collected and pellets

resuspended in 2% NH_4HCO_3. Chloroform was added to the pooled supernatant and mixed thoroughly before centrifuging to separate out the acetone fraction. The chloroform fraction was washed with NH_4HCO_3. Supernatants were pooled and acidified to pH 3. The acidified solution was partitioned twice with ethyl acetate. The ethyl acetate fraction was then partitioned with NH_4HCO_3 twice. The subnatants were pooled and acidified, extracted twice with ethyl acetate, and the pooled supernatants evaporated to approximately 0.5 ml under nitrogen. The fraction was strip-loaded onto prewashed paper and run for 3 hr in descending isopropanol : water : ammonia (10 : 1 : 1 v/v) with abscissic acid spots as standard.

Spots were marked out under UV light and corresponding areas cut out for elution overnight with 10% methanol : water mixture. The eluate was evaporated to dryness under nitrogen, washed with 1 ml petroleum spirit and the remaining film dried under nitrogen. The residue was dissolved in 1 ml ethyl acetate : methanol (1 : 1 v/v) and methylated with p-Tolysulphuryl methyl nitroso-amide until yellow. It was dried down under nitrogen and appropriate quantities of ethyl acetate were added prior to loading onto the GLC (Model 409 Beckman gas chromatograph).

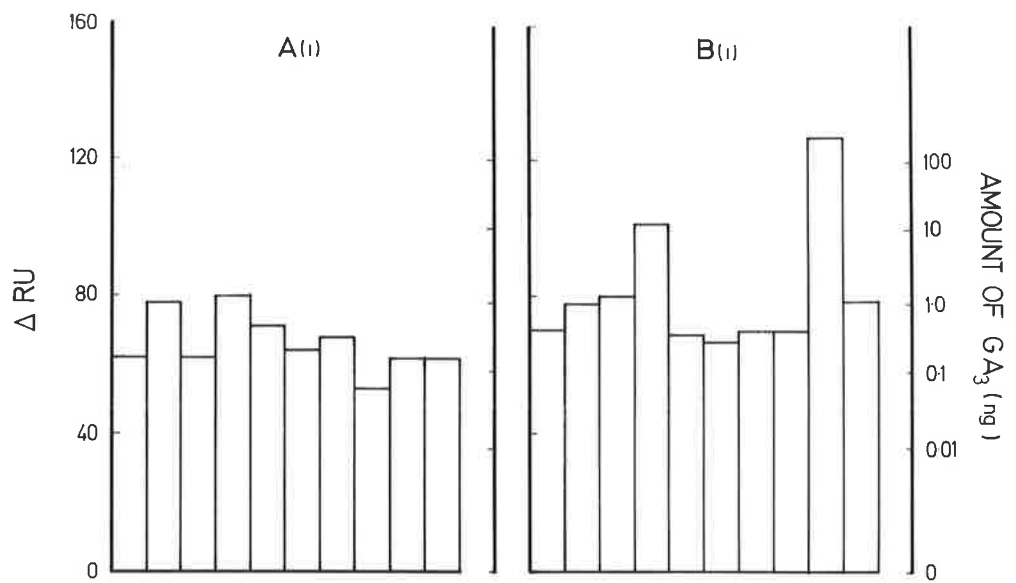
Results

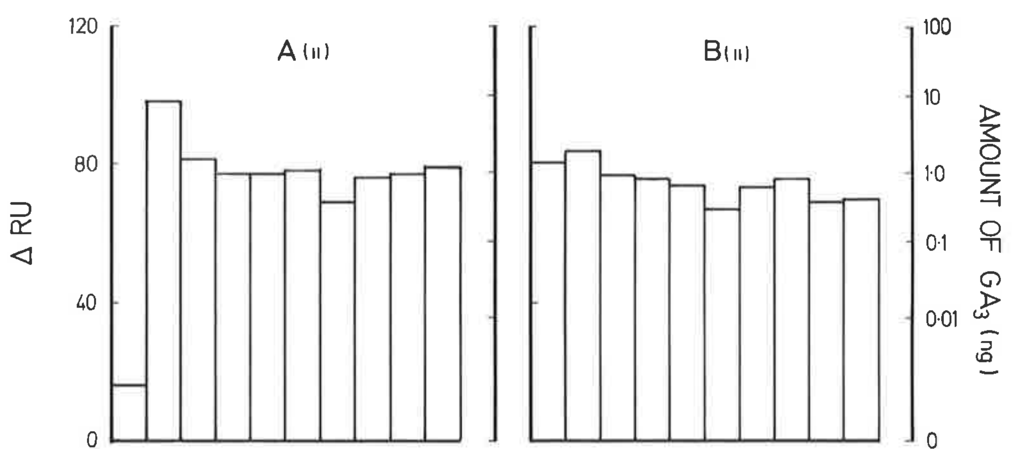
Gibberellins

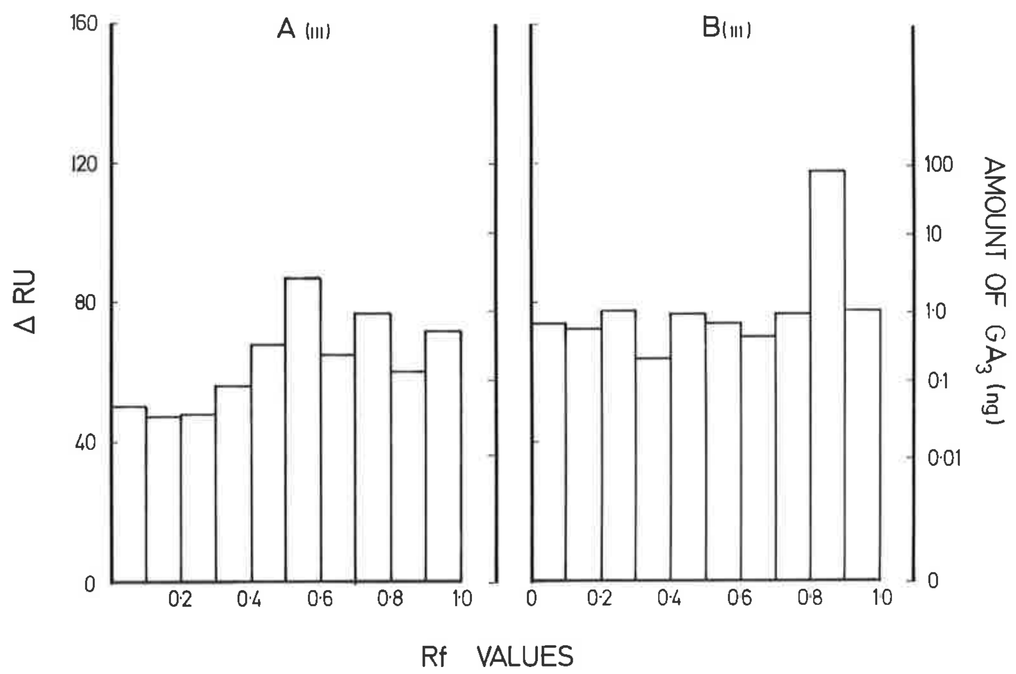
Fig. 15 A shows the gibberellin activity in the exudate of infected tomato plants. Fractions (i) and (iii) showed very low activity. Most activity was detected in fraction (ii) at the Rf of 0.1 - 0.3.

Figure 15.

Gibberellin activity from three fractions (i, ii, iii) extracted from the exudate of tomato plants infected with *M. javanica* (A) and non-infected plants (B).


A(i)
B(i)
160
120
80
40
0
Δ RU
100
10
1·0
0·1
0·01
0
AMOUNT OF GA$_3$ (ng)


A(ii)
B(ii)
120
80
40
0
Δ RU
100
10
1·0
0·1
0·01
0
AMOUNT OF GA$_3$ (ng)


A(iii)
B(iii)
160
120
80
40
0
Δ RU
100
10
1·0
0·1
0·01
0
AMOUNT OF GA$_3$ (ng)
0·2
0·4
0·6
0·8
1·0
0
0·2
0·4
0·6
0·8
1·0
Rf VALUES

Gibberellin extracted from the exudate of healthy plants is shown in Fig. 15 B. There was activity in fraction (i) in two zones, viz. 0.2 - 0.4 and 0.8 - 1.0 Rfs. Fraction (ii) showed low activity at the Rf of 0 - 0.2. Two zones of activity were detected in fraction (iii) at the Rfs of 0.3 - 0.5 and 0.8 - 1.0.

Fig. 16A shows gibberellin activity extracted from the galls. Activity was low and mostly in fraction (i) and (ii) whereas healthy root tissues (Fig. 16B) exhibited normal activity in all fractions.

Cytokinins

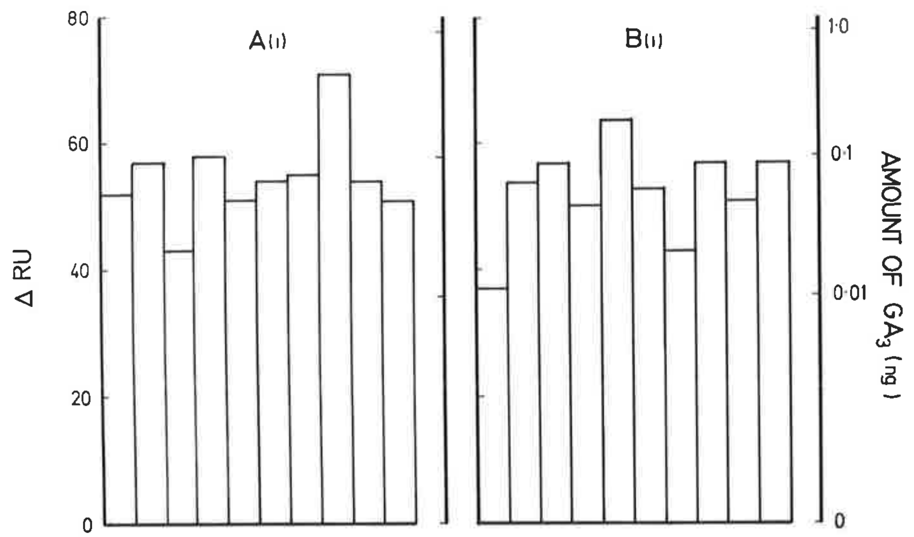
The soybean callus test indicated greater activity of cytokinin in exudate of healthy than in exudate from diseased plants (Fig. 17). Maximum response to callus growth occurred at lower levels of extract. Growth reduction of callus at high levels of extract concentration resulted from cytokinin being present at toxic concentrations. On the other hand, maximum activity of cytokinin in exudate from diseased plants was detected at higher levels of extract. There was less cytokinin in the galls as compared with the uninfected control.

Abscissic Acid

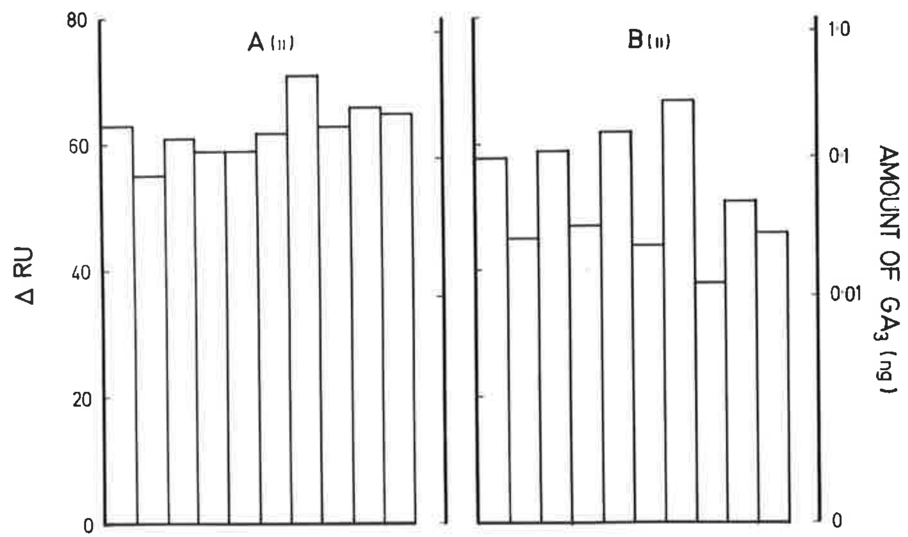
There was an increase in abscissic acid concentration in tomato plants infected with *M. javanica*. Values in both tops and roots were significantly different ($P < 0.05$), but the increase in tops was more pronounced as compared to the roots (Table 2).

Figure 16.

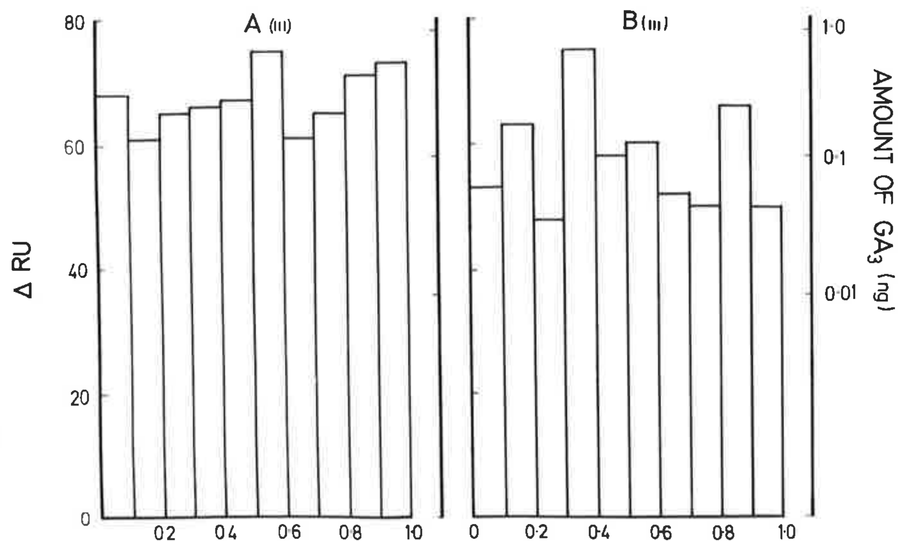
Gibberellin activity from three fractions (i, ii, iii) extracted from root galls (A) and healthy roots (B) of tomato plants infected with *M. javanica.*



A(i)
B(i)
Δ RU
AMOUNT OF GA3 (ng)



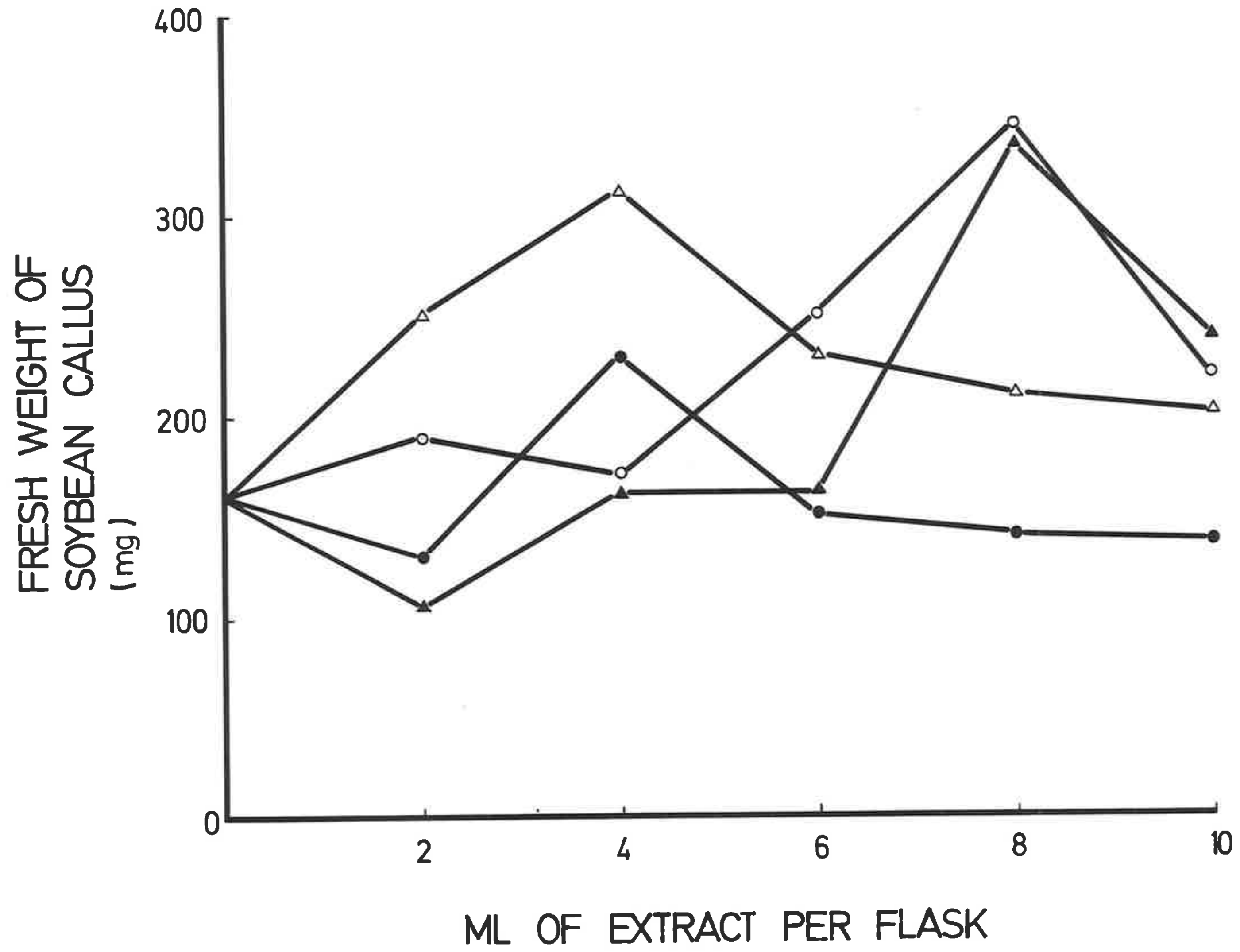
A(ii)
B(ii)
Δ RU
AMOUNT OF GA3 (ng)



A(iii)
B(iii)
Δ RU
AMOUNT OF GA3 (ng)
Rf VALUES

Figure 17.

Cytokinin activity extracted from healthy roots (o), galls (●), xylem exudate of healthy plants (Δ) and infected plants (▲) as measured by the soybean callus test.



400
300
200
100
0
FRESH WEIGHT OF
SOYBEAN CALLUS
(mg)
2
4
6
8
10
ML OF EXTRACT PER FLASK

Table 2.

Concentration of abscissic acid (ng/mg fresh weight) in tops and roots of tomato plants with and without *M. javanica*. Each value is the mean of three replicates.

	Roots	Tops
Infected	7.10	26.23
Control	5.72	15.92
L.S.D. $(P < 0.05)$	1.20	6.09

6. Discussion

Tomato plants infected with *M. javanica* developed pronounced galls and the internal structure of the root was modified in response to infection. When a larva reached a position where it could develop, it began to feed on cells adjacent to its head and these finally developed into giant cells (Christie, 1936). According to Bird (1962) giant cells require the repeated stimulus of the feeding nematode for their continued existence.

Giant cells in the vascular region of infected roots caused disruption of the xylem and irregular xylem-like elements developed. In areas where giant cells developed from parenchyma cells of the provascular strands, no cambium developed and consequently no secondary xylem vessels were formed. Studies with light and scanning electron microscopy showed that the cluster of giant cells was surrounded by a number of abnormal xylem elements which were not vertically placed as the normal ones but were disposed in a diffuse manner. If the infection were light the plant could compensate through the growth of its roots which, although galled, continue to grow, sometimes with increased branching. Infected plants have often been recorded as having heavier root systems than those of uninfected plants due to galling. On the other hand, a massive invasion of a root-tip can lead to the death of the root and the invading larvae.

The reason why plants quickly wilt in bright sunshine (Siddiqui, Rashid, Yunus and Ghouse, 1974) might be due to the injury caused when the nematodes enter the roots and to the disruption of the translocation system. But though secondary xylem is not produced in those sectors where giant cells develop, there are still masses of well-developed

secondary xylem in the other sectors. Thus, unless radial transport occurs readily, considerable resistance to the transport of water and nutrients will be produced because there are no long stretches of uninterrupted vessels. New rootlets are produced to replace those already attacked, and these in turn are attacked too, thus leading to exhaustion of the plant.

Although the infected individual root develops enormous amounts of xylem, heavily infected plants are generally seen wilting even under slight water stress, while the uninfected plants withstand the same conditions. The excess xylem produced as a measure to overcome the damage done due to a nematode attack, does not appear to help much in this direction. It appears that the abnormal xylem, though in enormous amounts, does not function as efficiently as normal xylem mainly due to its peculiar orientation which facilitates lateral rather than longitudinal conduction. Moreover, the abnormal structure and the smaller dimensions of the individual elements form a natural barrier to efficient conduction.

The difference in the rate of flow through galled and healthy plants and between the various levels of infection further support the hypothesis that abnormality of the xylem causes interference in sap flow in the vascular system. Melhus, Muncie and Ho (1924) found a similar relationship of galls to sap flow in the vascular system in crown gall (*A. tumefaciens*) on young apple trees and tomato plants. When forcing water through excised stem segments of tomato plants infected with *Verticillium* wilt, the resistance was found to be as much as 200 times that of healthy stems (Threlfall, 1959) and the resistance of turnips with late stages of oak wilt was found to

approach infinity (Gregory, 1971). Thus galled roots probably alter the water status of the host in the following ways: (i) absorption of water by the infected roots may be reduced due to inhibition and restriction of growth, and/or (2) sap flow in the vascular system may be impaired as a result of the abnormality of the xylem elements.

But the concentration of the elements potassium, phosphorus, magnesium, chlorine, sulphur and calcium determined by Phillips PW1540 x-ray fluorescence spectrophotometer did not differ significantly between the various levels of infection tested in either tops or roots although the most heavily galled roots tended to have an increased mineral content. This supports Walker and Wallace's (1975) work on the mineral content of French beans (*Phaseolus vulgaris*) infected by *M. javanica*. They reported that root-knot infection did not influence the mineral content of the tops although galling was clearly evident on the roots. The actual values were well within limits needed for normal growth.

However, stomatal closure in response to low water potential tends to inhibit the effects of increased root resistance on leaf water potential. The low water potentials induced by *M. javanica* could be attributed to an increased rate of transpiration and/or increased resistance to translocation of water from roots to leaves. The rate of transpiration is itself influenced by stomatal diffusibility which is in turn influenced by water potential. Following xylem disruption by *M. javanica*, increased root resistance contributes to decreased water potential. But comparison of the diffusive resistance

of infected and uninfected plants at the same water potential indicate that infected plants have higher diffusive resistance. In other words, although infection causes reduced water potentials in the leaves, this is offset to some extent by an increase in diffusive resistance which is greater than would be expected in an uninfected plant at the same water potential. This tends to maintain high water potential values in the leaves of infected plants. Duniway (1977) reported that although the transpirational demand and water potential relationships of plants can alter the influence of resistance to water uptake and turgor, his studies on *Phytophthora cryptogea* infected safflower plants indicate that resistance to water flow within infected plants must become very large before the resistance alone can cause lasting wilt symptoms in plants whose roots are well supplied with water. But even though very large increases in resistance are evidently required for lasting wilt symptoms to develop, it should be noted that all measurable increases in resistance to water uptake caused by infection are potentially damaging to the water relations and physiology of the infected hosts.

Since all plant physiological processes depend on water and if growth and development are to proceed normally, internal water stress must not develop within the plant tissues (Gates, 1972). Relatively low leaf moisture stress inhibits such processes as photosynthesis (Boyer, 1970) and transport to the shoot system of cytokinin synthesised in root-tips (Itai and Vaadia, 1971). As water deficits intensify, organelles and other cell components become disrupted (Todd, 1972). This causes further reductions in metabolic activity, which ultimately results in reduced growth (Duniway, 1973; Gates, 1972).

Hormonal changes are related to the water status of the intact plants (Itai and Vaadia, 1971); cytokinin decreased stomatal resistance by opening stomata and increased the resistance of the root to absorption of water. The general effect of the hormone is to reduce plant turgor. That cytokinin concentration decreased in nematode infected plants suggests that this is a mechanism for resisting water stress.

Thus, the observed variation in the growth hormones in the infected plants could be correlated with specific symptoms associated with the disease. Typical symptoms of infection are stunted growth, loss of yield, incipient wilting and decreased resistance to other pathogens. The stunted growth of the plant could be attributed to a decrease in gibberellin translocated to the shoot. This decrease in reserve gibberellin would result in a decreased supply of gibberellin necessary for normal growth of the plant. Wilting in the diseased plant could also be attributed to decreased gibberellin and cytokinin concentrations in the xylem exudate (Brueske and Bergeson, 1972). O'Bannon and Reynolds (1965) reported that cotton plants infected with the root-knot nematode absorbed less water than uninfected plants and consequently less water was available for translocation. The role of gibberellin and cytokinin in regulating transpiration is not fully understood, but cytokinin concentration in the xylem exudate is lowered in water-stressed plants (Itai and Vaadia, 1965). Livne and Vaadia (1965) found kinetin and gibberellin to stimulate transpiration. The action of the hormones may be to cause the opening of the stomata, thereby increasing transpiration and water uptake. In the diseased plants stomata would tend to open less because of the decrease in cytokinin and gibberellin precursors in the exudate. This would then

lead to decreased water uptake and wilting. Results on diffusive resistance to stomata and transpiration rate further support this notion.

Abscissic acid is a hormone responsible for alleviating the effects of water stress on plants (Hiron and Wright, 1973). Not only does it protect plants during periods of severe stress, such as drought, but it also appears to enable plants to withstand smaller stresses for long periods by a process of adaptation such as stomatal closure and reduced transpiration (Wright and Hiron, 1972; Wright, 1972; Hiron and Wright, 1973). Thus, the increase of abscissic acid upon exposure to infection is predictable in view of reports of the influence of abscissic acid on stomatal closure and consequent reduction of transpiration (Little and Eidt, 1968; Mittelheuser and van Steveninck, 1969; Jones and Mansfield, 1970; Mizrahi, Blumenfeld and Richmond, 1970) as well as enhancement of root exudation (Tal and Imber, 1971).

The association between the stunted growth of nematode-infected tomato plants and the reduction in gibberellin and cytokinin and an increase in abscissic acid is not evidence of a causal relationship. The production of growth inhibitors, or toxins by nematodes and/or the galled tissue might also contribute to the stunting effect. To understand the possible role of growth substances in disease development, their mode of action in normal plant metabolism clearly should be considered first. Not withstanding much study over a long period, many points are still far from clear.

The next phase in the nematode-plant association is the biochemical reaction of the plant cell to the nematode. It determines

the future fate of both nematode and plant. More important from the point of view of damage to the plant is the influence on plant cells and nematode of the chemical by-products resulting from the change in metabolic pattern within the plant tissues. Biochemical reactions initiated by the nematode may cause changes in the morphology, growth, differentiation and composition of the plant. Such changes may occur at a distance from the site of reaction, presumably because the chemical compounds responsible diffuse from cell to cell. The effects of some histologic changes caused by nematode attack may be so great as to be seen with the naked eye, whereas others may involve little disruption and be visible only under the microscope.

CHAPTER V

EFFECT OF INFECTION ON AMINO ACID COMPOSITION

A recent trend in characterising plant diseases is based on some aspect of the biochemistry of the host-pathogen relationship. Few studies have concerned changes in plant tissues associated with gall formation by plant parasitic nematodes. Higher concentrations of amino acids in *Meloidogyne*-induced root galls of tomato than in non-galled roots have been reported (Feldman and Hanks, 1964; Hanks and Feldman, 1963; Krusberg, 1961; Myuge, 1956; Owens and Novotny, 1960). Similarly, the presence of free amino acids was compared in healthy and *M. javanica*-infected jute roots and in the females of *M. javanica* (Saxena, 1972) by paper chromatography. Only a few of these studies present quantitative data.

Thus, the following studies were carried out with the objectives (1) to determine the quantitative changes in free and hydrolysed amino acids in roots of resistant and susceptible tomato plants infected with *M. javanica* and (2) to study the relative distribution of amino acids in the galls, females and eggs and egg sacs of *M. javanica*. Such information might provide a clue to the type of treatment that would allow the host to function normally, or nearly so, inspite of the presence of the pathogen.

Materials and Methods

1. Free and Hydrolysed Amino Acid Composition

Plant materials for these analyses were obtained from susceptible tomato cv. Early Dwarf Red and resistant cv. HES4242 inoculated with *M. javanica*. Egg masses were collected from heavily infected roots and dropped immediately into liquid nitrogen. Similarly, females were separated from the galls following the method of Hussey (1971).

All materials for amino acid determinations were kept at $-20^{o}C$ in liquid nitrogen until assay.

Extraction of Free Amino Acids

Plant material (500 mg fresh weight) was homogenised in 4.6 ml of methanol : chloroform : water for 3 min. at $0^{o}C$ to give 5 ml of extract. The actual volume used depended on the water content of the tissues, as an overall methanol : chloroform : water mixture of 2 : 1 : 0.8 (by volume) was aimed at. Nitrogen was bubbled through the slurry until it came to nearly room temperature. It was then centrifuged at 2,000 r.p.m. for 15 min. in a MSE major centrifuge in a $0^{o}C$ room. The supernatant was decanted off (filtered if necessary to remove floating material which was returned to the centrifuge tube) and stored under nitrogen. The residue was resuspended in 5 ml of methanol : chloroform : water, flushed with nitrogen and centrifuged. This procedure was repeated using a further one portion (5 ml) of methanol : chloroform : water mixture.

The residue was dried in a gentle stream of nitrogen for determination of protein-bound amino acids.

The methanol : chloroform : water extracts were pooled, their volume measured and sufficient chloroform and water added to give a methanol : chloroform : water ratio of 2:2:1.8 (by volume). The resultant mixture was shaken up, flushed with nitrogen, and centrifuged. The aqueous and chloroform phases were separated. The aqueous phase was evaporated to dryness in vacuo below $40^{o}C$ and dissolved in 2.0 ml citrate buffer (pH 2.2) for determination of the free amino acids. Amino acids were analysed on a Beckman 120C automatic analyser.

Hydrolysed Amino Acids

Samples of the dried residue were lyophilised and hydrolysed in 6N HCl at 110°C for 22 hours. These hydrolysates were dried under vacuum, then dissolved in 2.0 ml citrate buffer (pH 2.2) and analysed on a Beckman 120C automatic analyser.

Results

Free Amino Acids

Twenty-five amino acids were detected in galls, nematodes and in healthy and infected root tissues of tomato plants (Table 3). α-amino-n-butyric acid was not detected in the extract of eggs, and ethanolamine was detected only in root tissues. Only slight traces of methionine, histidine, arginine and ornithine were present in the healthy susceptible tissues, but were present in measurable quantities in the other extracts. Glutamic acid, proline and asparagine accounted for 40% or more of the free amino acids measured in the extracts of eggs. Though the absolute amounts of the free amino acids were higher in the infected tissues and eggs, the percentage composition of the free amino acids with few exceptions were quite similar. The greatest differences were observed with proline, alanine, asparagine and glutaminc.

Hydrolysed Amino Acids

Eighteen amino acids were detected in the protein hydrolysate of eggs, nematodes and root tissues of tomato (Table 4). However, certain amino acids were absent in some hydrolysates. Eggs lacked cysteine and methionine whereas the healthy susceptible roots lacked cysteine only. There was no correlation between the amount of an amino acid found in the free amino acid pool with the amount found in the protein hydrolysate. The only amino acid found in the protein hydrolysate

Table 3.

Absolute and relative amounts of free amino acids in eggs, nematodes and in root tissues with and without *M. javanica*.

Amino acids	Eggs		Nematodes		Infected susceptible root tissues		Healthy susceptible root tissues		Healthy resistant root tissues	
	nM/mg fresh wt	% of total	nM/mg fresh wt	% of total	nM/mg fresh wt	% of total	nM/mg fresh wt	% of total	nM/mg fresh wt	% of total
Aspartic acid	52.4	2.6	11.2	0.9	52.0	2.3	24.4	2.2	30.2	2.4
Threonine	65.8	3.2	39.4	3.4	42.8	1.8	25.4	2.3	27.6	2.2
Serine	88.0	4.3	49.2	4.2	66.6	2.9	56.6	5.1	59.2	4.7
Glutamic acid	270.6	13.3	52.2	4.5	224.8	9.8	73.4	6.6	109.2	8.6
Proline	586.6	28.8	86.2	7.4	568.2	24.9	26.6	2.4	108.2	8.5
Glycine	76.4	3.7	34.8	2.9	59.8	2.6	15.6	1.4	15.8	1.2
Alanine	132.6	6.5	45.4	3.9	123.8	5.4	14.2	1.3	26.2	2.0
Valine	61.2	3.0	61.6	5.3	48.4	2.1	25.2	2.2	27.0	2.1
Methionine	16.4	0.8	32.6	2.8	4.4	0.2	low	-	1.0	0.1
Isoleucine	38.2	1.9	55.0	4.7	36.6	1.6	11.6	1.0	15.0	1.2
Leucine	46.6	2.3	103.4	8.9	32.2	1.4	23.0	2.0	29.6	2.3
Tyrosine	37.8	1.8	22.6	1.9	27.0	1.2	5.6	0.5	4.4	0.3
Phenylalanine	16.6	0.8	18.3	1.5	23.0	1.0	6.0	0.5	10.0	0.8
Histidine	37.2	1.8	11.2	0.9	26.6	1.2	low	-	7.4	0.5
Lysine	13.2	0.6	6.6	0.5	12.0	0.5	9.8	0.9	9.8	0.8
Arginine	39.4	1.9	14.4	1.2	30.0	1.3	low	-	3.8	0.3
Taurine	4.8	0.2	0.8	0.1	11.0	0.5	4.0	0.4	9.6	0.7
α-amino-n-butyric acid	-	-	26.0	2.2	47.8	2.1	18.4	1.6	14.2	1.1
Asparagine	138.6	6.8	3.8	0.3	9.6	0.4	66.0	5.9	68.8	5.4
Glutamine	115.0	5.6	46.4	3.9	44.2	1.9	62.8	5.6	74.6	5.8
Cysthathionine	17.6	0.9	2.2	0.2	3.2	0.1	-	-	1.8	-
γ-aminobutyric acid	71.6	3.5	24.4	2.1	251.6	11.0	138.2	12.4	57.2	4.5
Ornithine	6.2	0.3	6.4	0.5	3.2	0.1	3.6	0.3	6.2	0.5
Ethanolamine	-	-	-	-	8.6	0.4	low	-	9.8	0.8
Ammonia	103.4	5.1	412.4	35.3	521.6	22.9	507.0	45.4	542.6	42.7
Total	2036.2	99.7	1166.5	99.5	2279.0	99.6	1117.4	100	1269.2	99.5

Table 4.

Absolute and relative amounts of hydrolysed amino acids in eggs, nematodes and in root tissues with and without *M. javanica*

Amino acids	Eggs		Nematodes		Infected susceptible root tissues		Healthy susceptible root tissues		Healthy resistant root tissues	
	nM/mg fresh wt	% of total	nM/mg fresh wt	% of total	nM/mg fresh wt	% of total	nM/mg fresh wt	% of total	nM/mg fresh wt	% of total
Aspartic acid	117.7	7.7	175.2	7.0	133.5	8.4	72.2	10.5	119.3	9.3
Threonine	119.4	5.4	105.9	4.3	75.3	4.8	34.7	5.0	59.1	4.6
Serine	110.1	4.9	120.5	4.8	92.9	5.9	52.5	7.7	80.2	6.2
Glutamic acid	182.8	8.2	331.5	13.3	154.4	9.8	64.6	9.4	118.3	9.2
Proline	153.9	6.9	165.2	6.6	87.9	5.6	37.5	5.5	59.5	4.6
Glycine	161.1	7.2	313.7	12.6	130.6	8.3	57.9	8.4	100.2	7.8
Alanine	120.5	5.4	159.2	6.4	98.6	6.2	52.2	7.6	94.5	7.4
Valine	78.9	3.5	109.4	4.4	76.6	4.9	42.7	6.2	74.8	5.8
Methionine	-	-	34.4	1.4	15.2	0.9	10.0	1.4	15.9	1.2
Isoleucine	62.4	2.8	89.6	3.6	56.7	3.6	29.5	4.3	54.8	4.3
Leucine	101.9	4.6	141.1	5.7	94.1	5.9	52.8	7.7	97.1	7.6
Tyrosine	48.7	2.2	48.4	1.9	53.7	3.4	12.2	1.8	29.2	2.3
Phenylalamine	115.0	5.2	58.5	2.3	66.4	4.2	23.2	3.4	50.8	3.9
Histidine	25.2	1.1	39.5	1.6	23.6	1.5	11.7	1.7	21.5	1.7
Lysine	103.3	4.6	125.8	5.0	94.1	5.9	46.9	6.8	82.8	6.4
Arginine	35.7	1.6	56.7	2.3	45.5	2.9	21.1	3.1	45.5	3.5
Cysteine	-	-	27.6	1.1	3.3	0.2	-	-	1.8	0.1
Ammonia	629.0	28.3	387.4	15.6	273.4	17.3	63.5	9.3	178.0	13.9
Total	2219.6	99.6	2489.5	99.9	1575.8	99.7	685.2	99.8	1283.3	99.8

but not detected as a free amino acid was cysteine. Tauric, asparagine, glutamine, α-amino-n-butyric acid, cystathione, γ-aminobutyric acid, ornithine and ethanolamine were detected in the free amino acid pool but not in the protein hydrolysate. If these were incorporated into the protein, they would probably have been destroyed or converted to other amino acids during hydrolysis and hence would not be detected in the protein hydrolysate. The absolute amounts of amino acids were higher in the eggs, nematodes and infected tissues but lower in healthy susceptible root tissues, but the percentage composition was quite similar with the exceptions of aspartic acid, glutamic acid and glycine.

2. Proline Accumulation

Associated with the increase in free amino acids in plants infected with *M. javanica* was the apparent accumulation of free proline. Accumulation of free proline has been reported by Owen and Specht (1966) in response to infection by root-knot nematodes. Similarly, grapefruit seedlings infected by *Radopholus similis* (Hanks and Feldman, 1963) and *Bidens tripartita* parasitised by *Longidorus africanus* (Epstein and Cohn, 1971) accumulated high concentration of proline. Alfalfa tissue galled by *Agrobacterium tumefaciens* showed a similar response (Seitz and Hochter, 1964). The accumulation of free proline in plants subjected to water deficit is well documented (Gusev and Gordon, 1968; Routley, 1966; Barnett and Naylor, 1966). Hence the question arises whether the accumulation of this amino acid in infected plants is also due to water stress caused through damage to the roots by pathogens. The following experiments attempt to examine this question by studies on tomatoes and cucumbers infected by *M. javanica* and to a lesser extent by *A. tumefaciens*.

Materials and Methods

Single tomato seedlings were grown in 10 cm plastic pots in John Innes potting mixture and maintained at field capacity by weighing to constant moisture for 40 days, after which 1-4 day old larvae of *M. javanica* were inoculated on to the surface of the soil at the base of each plant. Nematode densities were 0, 2,000, 4,000, 8,000 and 16,000 per pot with four replicates per treatment. Pots were arranged randomly on the glasshouse benches where temperatures fluctuated between 20°C and 30°C. Forty days after inoculation, the roots were carefully washed free of soil and blotted dry. Tops and roots were cut into 2-3 cm sections and pooled. Random samples were then taken for determinations of free proline.

Variation in proline concentration in infected tomato plants over an extended period of ten weeks was studied by inoculating the plants with 5,000 larvae of *M. javanica*. Uninfected plants were used as controls. Four plants from each treatment group were harvested every seven days when random samples of tops and roots were taken for proline determination. Distribution of proline was also determined in nematode eggs and egg sacs, females and in galls and in uninfected portions of the tomato roots.

To distinguish the effect of water stress from the effects of the nematode, infected and uninfected plants were maintained at 95% relative humidity. This was done by growing the plants in a growth chamber monitored to have 95% relative humidity and temperatures between 20°C and 25°C with 12 hours of light.

The concentration of free proline in cucumber plants (*Cucumis sativus* L.) infected with *M. javanica* was also studied. Seedlings were grown in 10 cm pots in John Innes potting mixture for thirty-five days, after which they were inoculated with 5,000 larvae of *M. javanica*. Uninoculated plants were used as controls. Forty days after inoculation, samples of tops and roots were taken for proline determination.

Proline was assayed in tomato seedlings infected with *A. tumefaciens*, either by inoculating the stems or by immersing the roots in a suspension of the bacterium. Galls were allowed to develop over a period of five weeks and then samples of the roots, tops and galls were taken for proline assay.

Concentrations of free proline were compared in susceptible and resistant tomato cultivars infected with *M. javanica*. Both susceptible (cv. Early Dwarf Red) and resistant (cv. HES4242) tomatoes were grown in 10 cm plastic pots for 40 days, after which they were inoculated with 8,000 larvae of *M. javanica*. Uninoculated plants were used as controls. Random samples of tops and roots were assayed for proline, forty days after inoculation.

Extraction and Measurement of Free Proline

A rapid method for estimating free proline has been developed (Singh, Paleg and Aspinall, 1973) based on the method of Troll and Lindsley (1955) for animal tissues. Similar extraction methods were applied to plant tissues. About 500 mg fresh weight of frozen tissue and about 1-2 gm of DeCalso Resin were placed in a Duall conical glass homogeniser at room temperature. Eight ml water were added to the

homogenate to break the stable emulsion formed during extraction. The mixture was then shaken and centrifuged. The upper aqueous layer was removed with a pipette into a boiling tube and 5 ml of glacial acetic acid and 5 ml fresh acidic ninhydrin reagent (125 mg ninhydrin : 3 ml CH_3COOH : 2 ml 6M orthophosphoric acid) were added. The mixture was then boiled for 45 min, cooled to room temperature, and shaken with a known volume of toluene (5-20 ml depending upon proline concentration). The optical density of the ninhydrin product dissolved in the toluene layer was measured at 520 nm and the proline concentration estimated from a standard curve.

Results

In tomato plants inoculated with *M. javanica* free proline increased with increase in nematode inoculum (Fig. 18), being higher in the roots than in the tops at all inoculum levels. Proline accumulated in infected roots and was maintained at a high level over a period of 10 weeks (Fig. 19). In infected roots, the highest levels of proline were obtained at the time of egg production. In tops from infected plants, the level of proline was high initially but then declined to about the level of uninfected plants. In infected roots, higher concentrations of proline occurred in the eggs and egg sacs than in the females and galls. Total amounts of proline were also relatively higher in the eggs and egg sacs (Table 5).

Under conditions of high relative humidity, as inoculum increased, proline again increased as in the previous experiments and furthermore it increased at a greater rate in the roots than in the tops (Fig. 20).

Figure 18.

The relationship between the number of nematodes in the inoculum and accumulation of free proline in roots (-----) and tops (——) of tomato plants 40 days after inoculation.

Each point is the mean of four replicates. Vertical lines indicate L.S.D. at $P < 0.05$.

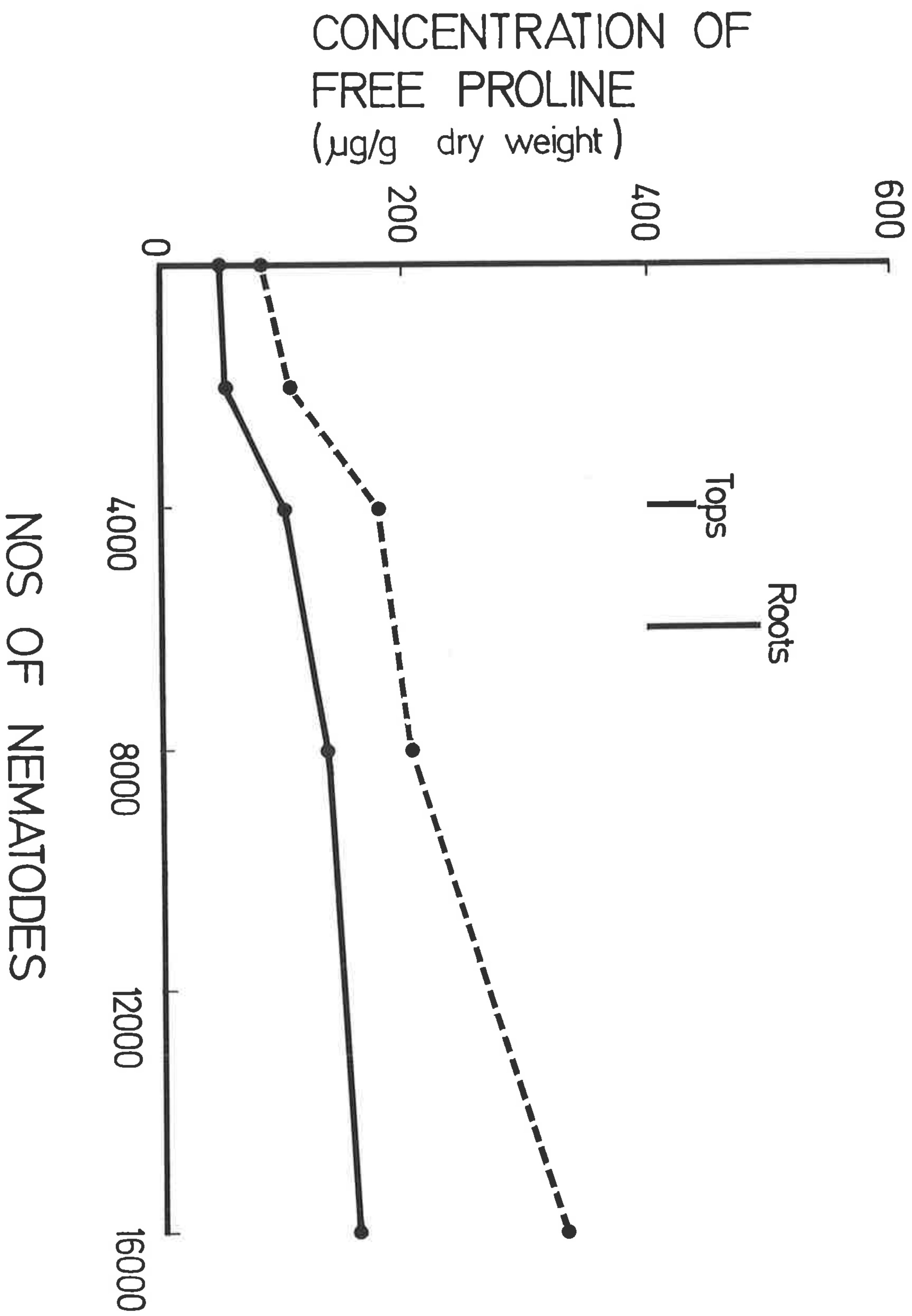

CONCENTRATION OF FREE PROLINE
(µg/g dry weight)
0
200
400
600
Tops
Roots
4000
8000
12000
16000
NOS OF NEMATODES

Figure 19.

The relationship between time of infection and accumulation of free proline in tomato roots (-----) and tops (———) infected with (●) *M. javanica* and uninfected (▲).

Each point is the mean of four replicates. Vertical lines indicate L.S.D. at $P < 0.05$. Arrow indicates initiation of egg laying.

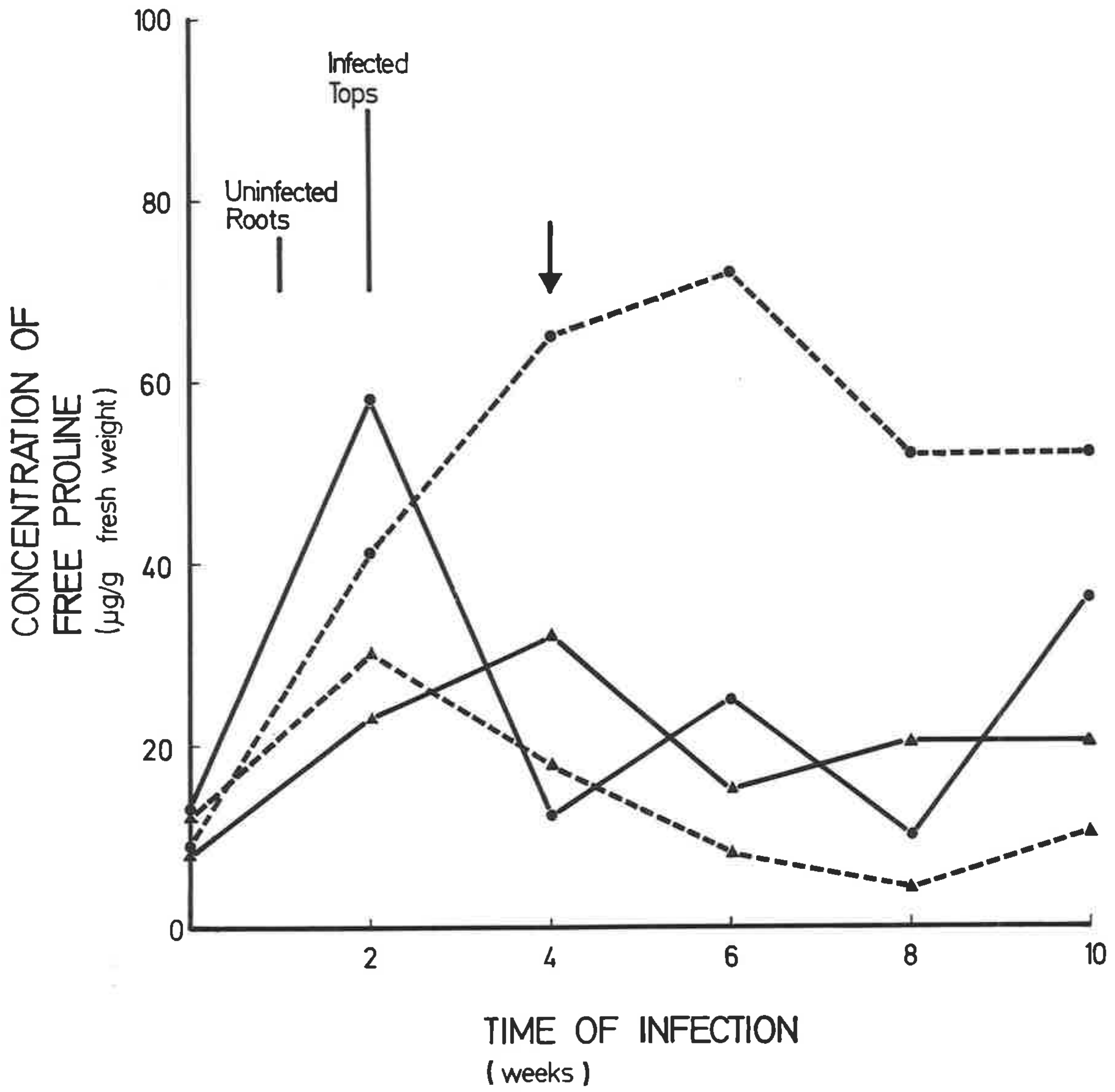

CONCENTRATION OF
FREE PROLINE
(µg/g fresh weight)
100
80
60
40
20
0
Infected
Tops
Uninfected
Roots
2
4
6
8
10
TIME OF INFECTION
(weeks)

Table 5.

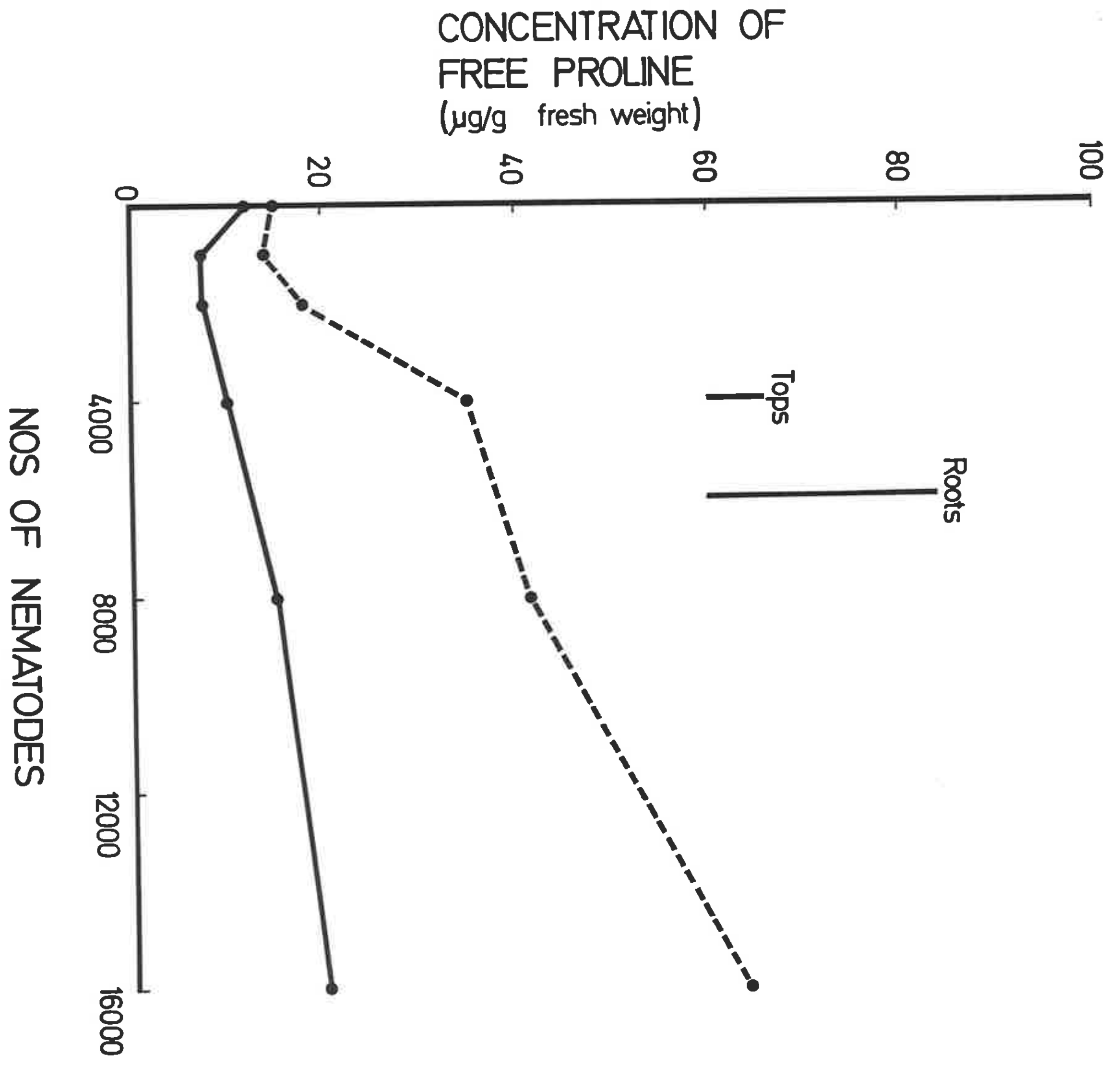
Distribution of free proline in roots of tomato plants infected with *Meloidogyne javanica*. Each value is the mean of four replicates.

	Fresh weight of sample (g)	Total amount of proline in sample (μg/g)	Concentration of proline μg/g fresh weight
Uninfected portions of roots	1.26	22.00	17.31
Galls	1.63	39.66	24.30
Eggs + Egg sacs	0.85	47.66	57.21
Females	0.62	2.33	11.49
L.S.D. ($P < 0.01$)	0.37	12.83	16.33

Figure 20.

The relationship between the number of *M. javanica* in the inoculum and the accumulation of free proline in roots (------) and tops (———) of tomato plants 40 days after inoculation. Plants were maintained at 95% R.H.

Each point is the mean of four replicates. Vertical lines indicate L.S.D. at $P < 0.05$.


CONCENTRATION OF
FREE PROLINE
(µg/g fresh weight)
0
20
40
60
80
100
Tops
Roots
NOS OF NEMATODES
4000
8000
12000
16000

When cucumber plants were infected with *M. javanica* there was a marked increase in proline in the roots. In the uninfected plants only small amounts of proline occurred in the tops and roots (Table 6).

Infection of tomato stems with *A. tumefaciens* resulted in a large discrete gall on the stem; infection of roots caused numerous small galls scattered over the whole root system. The concentration of proline in the stem gall was very high but because it was impossible to dissect away all root galls from unaffected root tissue, data for the galled root system are given (Table 7). The concentration of proline in the galled root system was higher than in the ungalled root system.

Higher concentrations of proline were obtained in healthy resistant than in healthy susceptible root tissues. With infection, there was an increase in proline concentration in the susceptible roots but the resistant cultivar did not show any significant increase (Table 8). There was no significant difference in the concentration of proline in tops of both cultivars with and without *M. javanica*.

3. Effects of Exogenous Amino Acids and Antimetabolites on Growth of *M. javanica* and tomato plants

A natural increase in free amino acids has been concomitant with an increase in resistance of some plants. Increase in resistance of soybean to stem canker (*Diaporthe phaseolorum*) was related to an increase in free amino acids (Benedict and Hilderbrand, 1958). Some success has been achieved in increased resistance to disease in specific hosts treated with certain amino acids, usually the DL-forms (Küc, Barnes, Daftsios and Williams, 1959; Papavizas and Davey, 1962; Van Andel, 1958; Zentmyer, Moje and Mircetich, 1962). Investigation on the use of systemic chemicals for control of spreading decline of citrus caused by

Table 6.

The influence of infection with *M. javanica* on free proline concentration (µg/g fresh weight) in cucumber plants. Each value is the mean of four replicates.

	Roots	Tops
Infected	32.29	5.12
Uninfected	2.90	3.22
L.S.D. ($P < 0.01$)	6.88	

Table 7.

Free proline concentrations (μg/g fresh weight) 5 weeks after inoculation with *A. tumefaciens* on stems or roots of tomato plants. Each value is the mean of four replicates. N.S. means not significantly different.

Site of inoculation	Stem galls	Tops	Roots	L.S.D. $P < 0.01$
Stem	430.1	138.1	31.71	67.83
Roots	-	163.7	191.3	N.S.

Table 8.

Proline concentration (μg/g fresh weight) in roots and tops of resistant and susceptible tomato plants with and without *M. javanica*. Each value is the mean of three replicates. N.S. means not significantly different.

		Roots	Tops
Susceptible	uninfected	32.69	34.25
	infected	58.49	32.65
Resistant	uninfected	34.30	34.42
	infected	36.17	34.18
L.S.D. ($P < 0.05$)		7.44	N.S.

Radopholus similis indicated restoration of plant vigour without control of the pathogen (Feldman and Hanks, 1962). The chemotherapeutic effect of amino acids or their antimetabolites on nematode infected plants has also received attention (Overman and Woltz, 1962; Prasad and Webster, 1967; Rao and Prasad, 1969; Evans and Trudgill, 1971; Reddy *et al.*, 1975 a and b). Thus, the following experiments attempt to study the possible use of certain amino acids for control of decline in growth caused by *M. javanica* in tomato plants.

Materials and Methods

In vitro Studies

Five egg masses of more or less uniform size, placed in 5 ml of different concentrations of the amino acids (100, 200, 400 µg/ml prepared in sterile distilled water) in screw-capped test-tubes and replicated three times, were incubated in the dark at 25°C and the number of larvae hatched was estimated using 1 ml counting dishes. Distilled water was used as controls. Similarly, 1,000 freshly hatched larvae placed in 5 ml of the different concentrations in screw-capped test-tubes and replicated three times, were incubated in the dark at 25°C for 48 hours. The larval suspension in each case was then transferred to a wire-gauze sieve containing two layers of facial tissue paper in a Petri dish holding sufficient water to remain in contact with the bottom of the wire gauze. After 24 hours, the larvae that had passed through the tissue paper into the Petri dish were counted.

Glasshouse Studies

Four-week old tomato seedlings grown in 3 cm x 15 cm plastic tubes containing sand were inoculated with 500 freshly hatched larvae

of *M. javanica*, by pouring the suspension around the base of the plant. The ten amino acids with their respective concentrations (25, 50, 100 mg/plant) dissolved in 5 ml of water were applied ten days after inoculation as a soil drench. Distilled water was used as controls. Shoot and root weights and ratio of shoot to root weights were recorded six weeks after inoculation to study the effect of amino acids on growth of tomato plants. Numbers of mature females were counted to determine the effect of the amino acids on growth and development of the nematode.

Results

Effects of Amino Acids on Hatching *in vitro*

All ten amino acids (including antimetabolites of proline) at all three concentrations inhibited larval hatching to varying degrees as compared to the control (Table 9). However, DL-asparagine inhibited hatching to the greatest extent; L-glutamine, L-trypthophan and hydroxyproline also showed extensive inhibition to larval hatching. The osmotic pressures corresponding to the concentration used were considered to have little effect on the nematodes.

Inhibitory Effect of Amino Acids to Larvae *in vitro*

In general, DL-amino acids (except DL-serine) and antimetabolites of proline (pipecolinic acid and picolinic acid) had some inhibitory effect on the second stage larvae of *M. javanica*, whereas L-amino acids (except L-serine) had no such effect (Table 10). Significant reduction in the number of larvae which survived was greatest in the highest concentration tested. The osmotic pressures corresponding to the concentration used probably had little influence on the nematodes.

Table 9

The effect of amino acids on hatching of *M. javanica in vitro*.

Each value is the mean of three replicates.

Treatment	Number of larvae hatched/5 egg masses in different concentrations			
	100 µg/ml	200 µg/ml	400 µg/ml	Mean
L-proline	650	300	231	393
L-glutamine	286	190	150	208
L-serine	835	560	260	551
L-trypthophane	220	150	150	173
DL-proline	696	678	358	577
DL-serine	513	485	278	425
DL-asparagine	100	81	71	84
Hydroxyproline	398	303	138	280
Pipecolinic acid	966	658	510	711
Picolinic acid	1006	946	918	957
Control	1035	1021	1015	1023

<u>L.S.D. at $P < 0.05$</u>

Between amino acids	90.5
Between concentrations	47.2
Amino acid X Concentrations	156.8

Table 10

The inhibitory effect of amino acids to 1-2 day old larvae of *M. javanica in vitro*. Each value is the mean of three replicates.

Treatment	Number of larvae which survived after 48 hours at different concentrations			
	100 µg/ml	200 µg/ml	400 µg/ml	Mean
L-proline	645	588	439	557
L-glutamine	657	537	520	571
L-serine	484	482	328	431
L-trypthophan	592	462	448	500
DL-proline	491	470	427	463
DL-serine	661	649	641	650
DL-asparagine	655	463	223	447
Hydroxyproline	550	359	344	418
Pipecolinic acid	372	384	351	369
Picolinic acid	467	372	300	379
Control	666	669	649	661

<u>L.S.D. at $P < 0.05$</u>

Between amino acids	65.7
Between concentrations	34.3
Amino acid X concentrations	113.8

Effects on Growth of *M. javanica* and tomato plants

Top growth of tomato plants infected with *M. javanica* was not affected by the amino acids tested, however fresh weight of root, ratio of fresh weight of tops to roots and number of females differed significantly ($P < 0.01$) between the treatments (Table 11). There was no significant difference between various concentrations of amino acids on either growth of plants or *M. javanica*. L-forms of amino acids (except L-glutamine) maintained growth of the tomato plants but the DL-forms and antimetabolites of proline (pipecolinic acid and picolinic acid) tended to inhibit growth. These amino acids were toxic at higher concentrations especially picolinic acid, DL-asparagine and hydroxyproline. When compared with controls the number of females decreased in plants treated with the DL-forms, whereas the L-forms (e.g. proline) supported a greater population of females.

4. Discussion

The females of *M. javanica* present in the infected roots account for less than 50% of the amino acid recovered in the analysis. Therefore, the direct contribution of the nematodes to the amino acid composition of the root is not significant and consequently the greater quantity of free amino acids in infected than in non-infected tissues indicates a plant response to infection. However, interpretation of biochemical changes in host-parasite complexes is difficult especially when obligate endo-parasites are involved (Howell and Krusberg, 1966; Saxena, 1972).

Table 11.

The effect of amino acids on the growth of *M. javanica* and tomato plants infected with *M. javanica*. Each value is the mean of three replicates.

Treatment	Concentration mg/plant	Fresh weight of tops (g)	Fresh weight of roots (g)	Ratio of tops to roots	Number of females
L-proline	25	4.53	1.36	3.33	310
	50	3.40	1.06	3.20	300
	100	2.26	0.70	3.22	376
L-glutamine	25	3.73	0.93	4.01	313
	50	3.93	0.86	4.56	136
	100	all plants died two weeks after treatment			
L-serine	25	4.83	1.30	3.71	250
	50	4.06	0.83	4.89	233
	100	3.03	0.63	4.80	113
L-trypthophan	25	4.56	1.26	3.62	253
	50	3.80	1.03	3.68	187
	100	2.70	0.90	3.00	80
DL-proline	25	4.43	1.00	4.43	223
	50	2.90	0.70	4.14	117
	100	3.53	0.96	3.68	276
DL-serine	25	4.33	1.03	4.20	163
	50	4.23	1.33	3.18	216
	100	2.46	0.53	4.64	40
DL-asparagine	25	4.50	1.10	4.09	130
	50	all plants died a week after treatment			
	100	" " " " " " "			
Hydroxyproline	25	3.53	0.83	4.25	76
	50	4.50	1.43	3.14	36
	100	all plants died three weeks after treatment			
Pipecolinic acid	25	3.40	0.96	3.54	286
	50	4.36	1.33	3.27	246
	100	3.53	1.20	2.94	183
* Picolinic acid	25	all plants died a week after treatment			
	50	" " " " " " "			
	100	" " " " " " "			
Control	25	4.03	1.26	3.19	320
	50	4.40	1.03	4.27	300
	100	4.43	1.33	3.33	300
L.S.D. ($P < 0.05$ between treatments		N.S.	1.09	1.19	87

N.S. means not significantly different.

* not included in the analysis of variance.

Increased levels of free amino acids after infection by root knot nematodes have been noted by Owens and Specht (1966), who found that free amino acids increased sharply in the galls of tomato. Increased levels of amino acids have also been found in galls of alfalfa caused by *Ditylenchus dipsaci* (Howell and Krusberg, 1966) and in galls initiated by *Longidorus africanus* on grapefruit roots (Epstein and Cohn, 1971). Changes in free amino acid content could occur in several ways. Howell and Krusberg (1966) noted that increases in levels of free amino acids in galled *versus* healthy tissues of alfalfa and pea could be due to increased translocation into the area of infection, increased rates of synthesis, decreased rates of translocation out of the gall, and/or decreased rates of breakdown. Since a large increase in bound amino acid also occurred, it seems doubtful that the greater increase in free amino acids in galls was due to hydrolysis of plant proteins by nematode enzymes.

Cytochemical analyses by Owens and Specht (1966) have shown that most of the increase in the free amino acids in tomato infected with root-knot nematodes occurred in the giant cells rather than in the galls. Owens and Specht (1966) considered that in tomato, accumulation of amino acids was due to synthesis in situ. Should this be true, the root knot resistant (HES 4242) cultivar must synthesize proportionately more amino acids than the susceptible variety. Results obtained both on hydrolysed and free amino acids of resistant and susceptible cultivars of tomato further support this view.

The amino acids that were identified in the eggs were obtained from the nematodes during passage through the nematode's body and were incorporated in high amounts into egg shells (Bird and McClure, 1976).

Similarly, incorporation of large amounts of amino acids into the eggshells were reported by Clarke *et al*. (1967) in *Heterodera rostochiensis*. The amino acids detected from the females were probably obtained from the infected tissues as the amino acids detected were present in both samples.

Some of the amino acids which increased with infection may be important in the host reaction to the nematode. Proline in some plants appears to be critically important in the formation of cell wall protein and the extensibility of the cell wall may well be controlled by the amount of hydroxylation of the proline residues in it (Thimann, 1972). In our analysis, thousand-fold or more increases in proline content of infected susceptible tissues were obtained especially during initiation of egg-laying. But accumulation of proline is also associated with some forms of stress other than infection. Plants such as ladino clover and Bermuda grass subjected to a water deficit accumulate proline in the tops (Gusev and Gordon, 1968; Routley, 1966; Barnett and Naylor, 1966), but tomato plants infected with *M. javanica* accumulate most proline in the roots. Furthermore, free proline was accumulated in infected tomato plants even when there was adequate soil water and a high atmospheric humidity. The results therefore suggest that proline accumulation is induced by factors other than water stress. The fact that cucumber, a plant that does not accumulate proline in response to water stress, does so when infected with *M. javanica*, supports this idea.

The hypothesis is proposed that high metabolic activity in the roots associated with giant cell and gall formation, and with egg productio[n] exerts a requirement for energy which is supplied by free proline manufactured in the leaves and translocated to the site of nematode activit[y]

The fact that *Agrobacterium tumefaciens* elicits a similar response in the galls tends to support the notion that sites of active cell division and growth induced by gall formation form metabolic sinks. Although McClure's (1977) studies with *Meloidogyne incognita* and Bird and Lovey's (1975) with *M. javanica* suggested the concept of a metabolic sink, contrary to Wallace's (1974) earlier work, the absence of data for the above-ground parts of the plants meant that the idea of a sink could only be applied to the root system. The present study removes this criticism and shows proline is more prevalent in roots than in tops, and in eggs and egg sacs and galls than in females and ungalled parts of the roots. It thus adds weight to the idea of a metabolic sink and to the view that the site of accumulation is closely associated with the developing nematode and adjacent plant tissues. The indication that proline accumulation reaches a maximum at about the time of egg production is also significant in this respect. Proline is a major constituent of the eggshells of *M. javanica* (Bird and McClure, 1976) and *Heterodera rostochiensis* (Clarke, Cox and Shepherd, 1967), so perhaps further research will delimit the site of proline requirement.

That proline accumulation is elicited by many factors other than a pathogen indicates the general nature of this response to stress. There are, of course, many other changes in the constituents of plants infected with root-knot nematodes; how proline fits into the general pattern of the physiology of the diseased plant is unknown. Free proline may merely be a by-product of little consequence to the plant or nematode. However, Roberts and Baba (1968) have proposed that proline may stimulate xylem production following injury, a suggestion supported by Cohn and Orion's (1970) observations that as well as increased levels of proline, xylem elements were scattered in the galled tissues of roots of grape and burr marigold plants infected with *Longidorus africans*. Furthermore,

the translocation of proline from the site of its manufacture in the leaves to the site of nematode activity in the roots suggests that this amino acid may be a ready source of energy.

It was interesting, therefore, to check whether addition or restriction of this amino acid, its antimetabolites and other amino acids would alter nematode activity and plant reaction to the infection. It is possible that the DL-forms of amino acids possess nematicidal activity, as results *in vitro* and *vivo* showed that these amino acids affect hatching, inhibit larvae and growth of the nematode as well as the plant. This hypothesis was further supported by the observation that the number of *Rotylenchulus reniformis* in infected soil was increased by the L-forms and decreased by the DL-form of proline (Rao and Prasad, 1969). But Epstein (1972) obtained opposite results after pretreating the plants first in DL-proline and L-proline antimetabolite, in which the number of nematodes increased significantly.

Overman and Woltz (1962) found that some amino acids inhibit reproduction of *Trichodorus christiei* and *Helicotylenchus nannus*, and decrease galling of tomato roots by *M. incognita*. Of eight amino acids tested by Prasad and Webster (1967) against four species of nematodes, DL-methionine and DL-alanine decreased the numbers of *Heterodera avenae* on oats, DL-ethionine and DL-alanine the numbers of *Ditylenchus dipsaci* on oats and DL-amino-butyric acid the numbers of *Aphelenchoides ritzemabosi* on lucerne. The present study, though not conclusive, did support this view as the DL-forms of amino acids tested have some degree of inhibition on growth of *M. javanica* and tomato plants especially at high concentrations. Such chemicals due to their biological specificity, would block specific metabolic reactions

and cause the nematodes to 'starve in the midst of plenty' (Overman and Woltz, 1962). Hence further research might usefully attempt to ascertain whether certain amino acids could possibly serve as a nematicidal role in agriculture, as they accumulate in the giant cells of nematode infected plants (Owens and Novotny, 1960; Peacock, 1960). Moreover the use of structural variants (analogues) of natural metabolites of plants and animals to control populations of undesired species has been poorly developed in the field of agriculture. Therefore, the ultimate resolution of nematode control may be the development of chemical analogues which may serve as antimetabolites in the biological system of nematodes without seriously interfering with the normal metabolism of the host plant.

CHAPTER VI

GENERAL DISCUSSION AND CONCLUSIONS

The research described in this thesis supports the view that the association between plant and nematode is such that a change in the physiology of either organism produces an appropriate change in the other to increase the chance of survival of both organisms. Furthermore the nature of this association is influenced by environment which affects each organism and their interactions.

The association between nematode and plant can be described as follows: The nematode always penetrates at the root tip thereby inhibiting the production of gibberellic acid and cytokinins. Consequently plant growth is reduced to an extent determined by the numbers of larvae invading the root. With low infection levels root tips often recover and grow on and those that are killed are replaced by the regeneration of new ones, so hormonal levels might be expected to be restored except that by this time disruption to the vascular system, particularly xylem elements, has occurred. Thus translocation of hormones from the roots is restricted. In addition to hormones, however, water and nutrient supply to the tops is also impaired. Furthermore, restricted root growth further reduces the ability of the plant to absorb water and nutrients from the soil. In my experiments weights of infected root systems increased substantially due to galling; a better physiological parameter would have been surface area of roots or area of absorptive surface both of which probably decline in infected roots.

The reduced supply of water and nutrients to the tops could have several consequences, the most obvious of which is a reduced rate of metabolism and growth. However the extent of such a reduction depends on the availability of water and nutrients to the plant. Apart from the restricted root system already mentioned, a lack of water and nutrients in the soil would exacerbate the effects already induced in the plant's vascular system by the nematode. Clearly, environment can never be excluded from the relationships between nematode and plant that result in disease. The initial density of nematodes infecting the roots is a further important factor in this complex system.

A further consequence of a restricted water supply is to lower the water potential of the leaves. In uninfected plants such a stress causes the stomata to close thereby reducing diffusivity and conserving water. The same appears to occur in nematode-infected plants except that the response is stronger, i.e. at the same water potentials in the leaves the stomatal diffusivity of infected plants is lower than in uninfected plants. Such a response tends to maintain the integrity of the infected plant by conserving water, to increase its tolerance to infection and accounts in part for the observation that, in the experiments described in this thesis, infected plants showed little signs of wilting or stunting. The mechanism whereby the infected plant achieves this regulation is not understood but it is possible that restriction of the supply of such hormones as cytokinins and gibberellins to the tops and the production of abscissic acid in the tops are important contributory factors.

A further aspect of the research described in this thesis concerns the translocation of materials downwards in the phloem. Two immediate questions arise: if disruption of the vascular system reduces upward translocation why does it not reduce downward translocation? It is possible that the phloem is not affected by the nematode to the same extent as the xylem, but this requires further study. The second question concerns the notion of a metabolic sink in the roots. What is the role of proline in the plant-nematode relationship? If it is an important constituent of the nematode egg or an essential source of energy it is tempting to speculate that control of the nematode might be achieved by developing analogues of proline and other amino acids which, although not affecting plant growth, might retard growth of the nematode, inhibit egg production or hatching or increase the tolerance of the host to infection. Further research along these lines might be fruitful.

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PUBLICATIONS

1823

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Changes in free proline following infection of plants with either *Meloidogyne javanica* or *Agrobacterium tumefaciens*

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Infection of tomato plants by either *Meloidogyne javanica* or *Agrobacterium tumefaciens* resulted in accumulation of free proline in galls whether they were on roots or stems. The concentration of free proline increased with increasing density of inoculum of nematodes and varied with time, the highest concentration occurring at the time of egg production. Water stress was not necessary for the accumulation of proline as it accumulated in infected tomato in the absence of water stress, and in infected cucumber plants which normally do not accumulate proline under water stress. The concentration of free proline was highest in eggs and egg sacs and in the galls as compared to uninfected portions of roots of infected tomato plants.

INTRODUCTION

Galls induced in tomato roots in response to infection by root knot nematodes contain large amounts of free amino acids (particularly proline), protein and nucleic acids [*10*]. Similarly, grapefruit seedlings infected by *Radopholus similis* (Cobb) Thorne [*8*] and *Bidens tripartita* L. parasitized by *Longidorus africanus* Merny [*6*] accumulate high concentrations of proline. Alfalfa tissue galled by *Agrobacterium tumefaciens* (Smith & Townsend) Conn shows a similar response [*13*]. The accumulation of proline in plants subjected to water deficit is well known [*1, 7, 12*]. But some cucurbits, including cucumbers (*Cucumis sativus* L.), are known not to accumulate proline even under severe water stress (D. Aspinall, private communication). Hence the question arises whether the accumulation of this amino acid in infected plants may be due to water stress caused through damage to the roots by pathogens. This paper examines this question by studies on tomatoes (*Lycopersicon esculentum* Mill. cv. Early Dwarf Red) and cucumbers infected with *Meloidogyne javanica* (Treub) Chitwood and tomatoes infected with *A. tumefaciens*.

MATERIALS AND METHODS

Single tomato seedlings (*Lycopersicon esculentum* cv. Early Dwarf Red) were grown in 10 cm plastic pots in John Innes potting mixture and maintained at field capacity by weighing to constant moisture for 40 days, after which 1- to 4-day-old larvae of *Meloidogyne javanica* were inoculated on to the surface of the soil at the base of each

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1824

plant. Nematode densities were 0, 2000, 4000, 8000 and 16 000 per pot with four replicates per treatment. Pots were arranged randomly on the glasshouse benches where temperatures fluctuated between 20 °C and 30 °C. Forty days after inoculation, the roots were washed free of soil and blotted dry. Tops and roots were cut into 2 to 3 cm sections and pooled. Random samples were then taken for determination of free proline.

Variation in proline concentration in infected tomato plants over an extended period of 10 weeks was studied by inoculating the plants with 5000 larvae of *M. javanica*. Uninfected plants were used as controls. Four plants from each treatment group were harvested every 7 days when random samples of tops and roots were taken for free proline determination. Distribution of proline was also determined in nematode eggs and egg sacs, females and in galls and uninfected portions of the tomato roots.

To distinguish the effect of water stress from the effects of the nematode, infected and uninfected plants were maintained at 95% relative humidity. This was done by growing the plants in a growth chamber monitored to have 95% relative humidity and temperatures between 20 °C and 25 °C with 12 h of light.

The concentration of free proline in cucumber plants (*Cucumis sativus*) infected with *M. javanica* was also studied. Seedlings were grown in 10 cm plastic pots in John Innes potting mixture for 35 days, after which they were inoculated with 6000 larvae of *M. javanica*. Uninoculated plants were used as controls. Plants were harvested 40 days after inoculation when random samples of tops and roots were taken for free proline determination.

Proline was assayed in tomato seedlings infected with *Agrobacterium tumefaciens*, either by inoculating the stems or by immersing the roots in a suspension of the bacterium. Galls were allowed to develop over a period of 5 weeks and then samples of the roots, tops and galls were taken for proline assay.

All samples for proline assay were wrapped in aluminium foil, immediately frozen in liquid N_2 and stored at −20 °C until assay.

Data were statistically analysed by analysis of variance. The results of the experiments are shown graphically as untransformed data.

Extraction and measurement of free proline

A rapid method for estimating free proline has been developed [*14*] based on the method of Troll & Lindsley [*15*] for animal tissues. Similar extraction methods were applied to plant tissues. About 500 mg fresh weight of frozen tissue and about 1 to 2 g of Decalso Resin were placed in a Duall conical glass homogenizer at room temperature. Eight millilitres of water was added to the homogenate to break the stable emulsion formed during extraction. The mixture was then shaken and centrifuged. The upper aqueous layer was removed with a pipette into a boiling tube and 5 ml of glacial acetic acid and 5 ml fresh acidic ninhydrin reagent (125 mg ninhydrin : 3 ml CH_3COOH : 2 ml 6 M orthophosphoric acid) were added. The mixture was then boiled for 45 min, cooled to room temperature and shaken with a known volume of toluene (5 to 20 ml depending upon proline concentration). The optimal density of the ninhydrin product dissolved in the toluene layer was measured at 520 nm and the proline concentration estimated from a standard curve.

RESULTS

In tomato plants inoculated with *M. javanica* free proline increased with an increase in nematode inoculum (Fig. 1), being higher in the roots than in the tops at all inoculum levels. Proline accumulated in infected roots and was maintained at a high level over a period of 10 weeks (Fig. 2). In infected roots, the highest levels

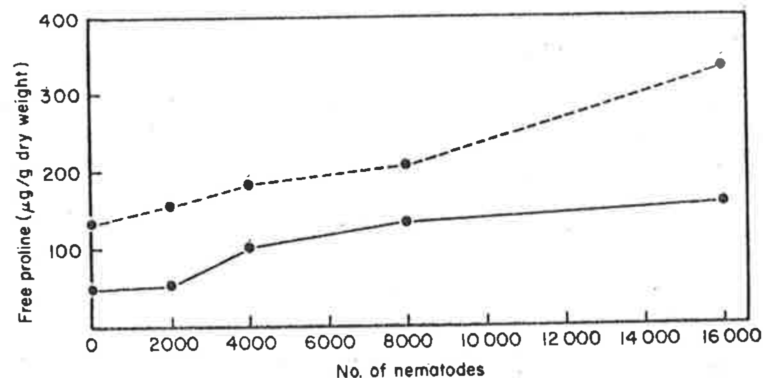

Fig. 1. The relationship between the number of nematodes in the inoculum and subsequent amounts of free proline in roots (- - -) and tops (——) of tomato plants 40 days after inoculation with *M. javanica*. L.S.D. ($P<0{\cdot}05$) for roots = 87·1, for tops = 34·0. Each point is the mean of four replicates.

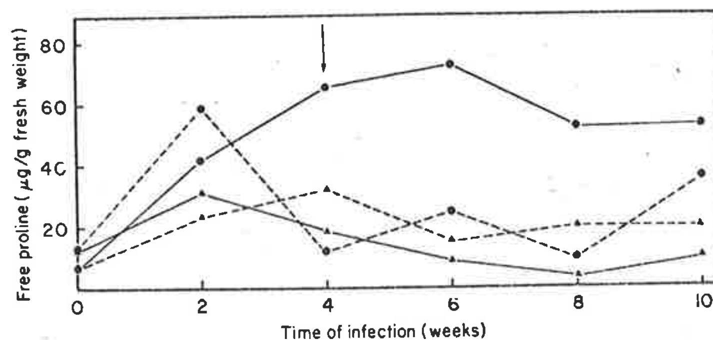

Fig. 2. The relationship between time of infection and accumulation of free proline in tomato roots (——) and tops (- - -) infected with (●) *M. javanica* and uninfected (▲). L.S.D. ($P<0{\cdot}05$) for infected tops = 20·9, for uninfected roots = 6·94. Values for infected roots and uninfected tops are not significant. Each point is the mean of four replicates. Arrow indicates initiation of egg production.

of proline were obtained at the time of egg production. In tops from infected plants, the level of proline was high initially, but then declined to about the level of uninfected plants. In infected roots, higher concentrations of proline occurred in the eggs and egg sacs than in the females and galls. Total amounts of proline were also relatively higher in the eggs and egg sacs (Table 1).

Under conditions of high relative humidity, as inoculum increased, proline again increased as in the previous experiments and furthermore it increased at a greater rate in the roots than in the tops (Fig. 3).

1825

When cucumber plants were infected with *M. javanica* there was a marked increase in proline in the roots. In the uninfected plants, only small amounts of proline occurred in the tops and roots (Table 2).

TABLE 1

Distribution of free proline in roots of tomato plants infected with M. javanica[a]

	Fresh weight of sample (g)	Total amount of proline in sample (μg)	Concentration of proline (μg/g fresh weight)
Uninfected portions of roots	1·26	22·00	17·31
Galls	1·63	39·66	24·30
Eggs+egg sacs	0·85	47·66	57·21
Females	0·62	2·33	11·49
L.S.D. $P<0·001$	0·37	12·83	16·33

[a] Each is the mean of four replicates.

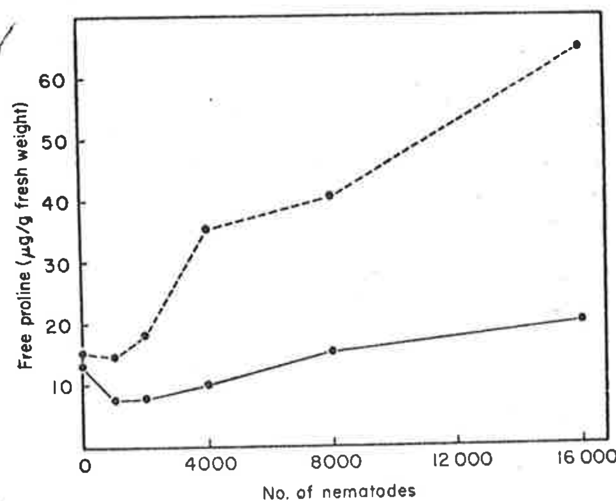

FIG. 3. The relationship between the number of *M. javanica* in the inoculum and the accumulation of free proline in roots (- - -) and tops (——) of tomato plants 40 days after inoculation. Plants were maintained at 95% r.h. L.S.D. ($P<0·05$) for roots = 42·4, tops = 6·3. Each point is the mean of four replicates.

Infection of tomato stems with *A. tumefaciens* resulted in a large discrete gall on the stem; infection of roots caused numerous small galls scattered over the whole root system. The concentration of proline in the stem gall was very high but because it was impossible to dissect away all root galls from unaffected root tissue, the concentration of proline in the galled root system was higher than in the ungalled root system (Table 3).

DISCUSSION

Infection of tomato plants by *M. javanica* is associated with accumulation of free proline. Such an accumulation might be caused by water stress following disruption

Table 2

The influence of infection with M. javanica *on free proline concentration (μg/g fresh weight) in cucumber plants*[a]

	Roots	Tops
Infected	32·29	5·12
Uninfected	2·90	3·22
L.S.D. $P < 0{\cdot}01$	6·88	

[a] Each value is the mean of four replicates.

Table 3

Free proline concentrations (μg/g fresh weight) after 5 weeks in tomato plants inoculated with A. tumefaciens *on stems or roots*[a]

Site of inoculation	Stem galls	Tops	Roots	L.S.D. $P < 0{\cdot}01$
Stem	430·1	138·1	31·71	67·83
Root	—	163·7	191·3	N.S.

Each value is the mean of four replicates. N.S. means not significantly different.

of xylem and reduced translocation of water from roots to top when giant cells are formed in response to infection by the nematode. However, plants such as ladino clover and Bermuda grass subjected to a water deficit accumulate proline in the tops [*1*, *7*, *12*], rather than in the roots whereas tomato plants infected with root knot nematodes accumulate most proline in the roots. Furthermore, free proline was accumulated in infected tomato plants even when there was adequate soil water and a high atmospheric humidity. The results, therefore, suggest that proline accumulation is induced by factors other than water stress. The fact that cucumber, a plant that does not accumulate proline in response to water stress, does so when infected with *M. javanica*, supports this idea.

The hypothesis is proposed that high metabolic activity in the roots associated with giant cell and gall formation, and with egg production, exerts a requirement for energy which is supplied by free proline manufactured in the leaves and translocated to the site of nematode activity. The fact that *A. tumefaciens* elicits a similar response in the galls tends to support the notion that sites of active cell division and growth induced by gall formation form metabolic sinks. Although McClure's [*9*] studies with *Meloidogyne incognita* and Bird & Lovey's [*2*] with *M. javanica* suggested the concept of a metabolic sink, contrary to Wallace's [*6*] earlier work, the absence of data for the aboveground parts of the plants meant that the idea of a sink could only be applied to the root system. The present study removes this criticism and shows proline is more prevalent in roots than tops, and in eggs and egg sacs and galls than in females and ungalled parts of the root. It thus adds weight to the idea of a metabolic sink and to the view that the site of accumulation is closely associated with the developing nematode and adjacent plant tissues. The indication that proline accumulation reaches a maximum at about the time of egg production is also significant in this respect. Proline is a major constituent of the egg shells

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of *M. javanica* [*3*] and *Heterodera rostochiensis* [*4*], so perhaps further research will further delimit the site of proline requirement.

That proline accumulation is elicited by many factors other than pathogens indicates the general nature of this response to stress. There are, of course, many other changes in the constituents of plants infected with root knot nematodes; how proline fits into the general pattern of the physiology of the diseased plant is unknown. Free proline may merely be a by-product of little consequence to the plant or nematode. However, Roberts & Baba [*11*] have proposed that proline may stimulate xylem production following injury, a suggestion supported by Cohn & Orion's [*5*] observations that as well as increased levels of proline, xylem elements were scattered in the galled tissues of roots of grape and burr marigold plants infected with *Longidorus africanus*. Furthermore, the translocation of proline from the site of its manufacture in the leaves to the site of nematode activity in the roots suggests that this amino acid may be a ready source of energy. Hence further research might usefully attempt to ascertain whether proline is essential for the development of giant cells and galls, as well as for nematode reproduction.

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Submitted for Publication:
Physiological Plant Pathology.

1

2 Water Relations of Tomato (*Lycopersicum esculentum* Mill. cv. Early Dwarf

3 Red) infected with *Meloidogyne javanica* (Treub), Chitwood

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1 Abstract

2 Infection of tomato plants by *Meloidogyne javanica* resulted in
3 increased resistance of the root system to water uptake and translocation
4 due to abnormality of the xylem elements. A change in the root resistance
5 to water uptake and/or transpirational behaviour appeared to account for
6 the low water potential values in the infected plants. There was no
7 difference in the rate of transpiration between infected and non-infected
8 plants; however, diffusive resistance increased in infected plants as
9 infection progressed and was higher than that in healthy plants. Therefore,
10 the inhibition of water uptake and of translocation, together with some
11 inhibition of stomatal opening, probably explains why infected plants did
12 not show any marked foliar symptoms throughout the experimental period.

1 ## Introduction

2 All plant physiological processes depend on water and if growth and
3 development are to proceed normally, internal water stress must not develop
4 within the tissues (5). Low moisture stress in leaves inhibits such
5 processes as photosynthesis (2) and transport to the shoot system of
6 cytokinins synthesised in root tips (6), thereby causing reductions in
7 metabolic activity which ultimately result in reduced growth (4), (5).
8 The influence of pathogens in altering water potential gradients is not
9 well understood.

10 Because the movement of water through a plant system is determined in
11 part by the sum of the resistances encountered in the pathway, and since
12 resistance to water movement is greatest in the intervening tissue between
13 the root epidermis and the xylem (9), appreciable changes in water trans-
14 port can result from changes in the resistance of roots to water flow.

15 Yield reductions of a wide variety of crops result from root infesta-
16 tions by the root knot nematode, *Meloidogyne javanica* (Treub), Chitwood.
17 Stunting resulting from root-knot infection is often associated with root
18 tissue damage. Infected plants have been reported to appear more susceptible
19 to wilt than healthy plants during periods of slight water stress, although
20 the effect of *M. javanica* on host-water relations has never been
21 quantitatively measured. The purpose of this investigation was to
22 determine the effect of *M. javanica* on water-relations of tomatoes
23 (*Lycopersicum esculentum* Mill. cv. Early Dwarf Red).
24

1 Materials and Methods

2 Galls of various sizes and of varying age were collected from tomato
3 plants infected with *M. javanica* and fixed in F.A.A. for histological
4 studies. After three days in fixative, the galls were washed with tap
5 water and dehydrated in the tertiary butyl alcohol series. They were
6 embedded in paraffin and then sectioned on a sliding microtome at a thickness
7 of 15 μ in transverse and longitudinal planes. The sections were stained in
8 safranin and fast green. After dehydration in an ethanol series, temporary
9 mounts were prepared for microscopic examinations.

10 To study the effect of infection on the resistance of the root to
11 water flow, single tomato seedlings were grown in John Innes potting
12 compost in 10cm pots for forty days, after which larvae of *M. javanica*
13 were inoculated into the soil at the base of each plant. Inoculum densities
14 used were 0, 500, 1,000, 2,000, 5,000 or 10,000 larvae. The plants were
15 then subjected to high (25%), medium (16%) and low (8%) moisture contents,
16 three days after inoculation. Moisture in each system was kept constant by
17 weighing to constant weight. The pots were randomly arranged in the growth
18 cabinet with temperatures of 20°C in the dark (12 hr/day) and 25°C in the
19 light (12 hr/day). Forty days after inoculation, the plants were cut off
20 approximately 4cm from the soil level and the root resistance was measured
21 using the modified apparatus of Melhus *et al.*(8) designated as a 'Bomb
22 Calorimeter'. The pressure required to drive the first drops of water to
23 the cut surface of the stem was taken to be directly proportional to the
24 resistance of the root system.

25 For determination of diffusive resistance of stomata and water potential
26 of leaves, tomato plants grown in 12.5cm pots were randomly arranged in the
27 growth cabinet with temperatures of 20°C in the dark (12 hr/day) and 25°C in
28 the light (12 hr/day). Each plant was inoculated with 6,000 larvae of *M.*
29 *javanica* and uninoculated plants were used as controls. Measurements commenced

one week after inoculation and continued at intervals of seven days for a period of 8 weeks. Soil was brought to field capacity at the beginning of each measurement. The diffusive resistance of the abaxial leaf surface of healthy and infected tomatoes was measured with an aspirated diffusive porometer (3). Measurements were taken four hours after saturation of the rooting medium with water in the light period. Data collected from diffusive resistance measurements of the third pair of true leaflets at similar physiological ages were converted to sec cm^{-1}. The same leaf was then detached and immediately placed into a peltier-cooled thermocouple psychrometer to determine its water potential (1). The leaves were wrapped around a wire mesh insert protecting the thermocouple and pushed into the psychrometer chamber, which was then stoppered. The chamber was placed in a water bath (25°C ± 0.01°C) and allowed to equilibrate for at least 2 hours before reading the thermocouple output. The water potential was calculated by comparing the recorded deflections with the deflections obtained from a graded series of sodium chloride solutions.

Transpiration rate was studied by gravimetric analysis involving growing plants in 500ml waxed paper cups containing sand. Each plant was inoculated with 1,000 larvae of *M. javanica*. Uninoculated plants were used as controls. A closed system surrounding the roots was provided as follows: a drain hole was made in the bottom of the cup, and a watering tube inserted into the sand. The surface was then covered with polystyrene to prevent water loss from the soil surface and pots. Each cup was inserted into a second cup and the watering tube sealed with clay. At the beginning of each measurement the outside cup was removed and Hoagland's solutions added through the watering tube until the sand was saturated. The system was allowed to drain for 30 mins and resealed. The difference in weight of the saturated system after 24 hours was expressed as the total rate of the whole-plant transpiration (g/24 hours).

All the results were analysed statistically by regression analysis and are represented graphically as untransformed data.

Results

Histological studies showed that giant cells were formed from the parenchyma cells of the vascular system in response to infection which also inhibited cambium formation, hence no secondary xylem was formed (Fig. 1A). Normal conducting vessels were present away from the infection sites. The cluster of giant cells was usually surrounded by a large number of abnormal xylem-like elements (Fig. 1B). The abnormal xylem was not arranged along the longitudinal axis of the root but was disposed in a diffuse manner, and furthermore its elements were shorter and narrower than normal ones. Although vessels of large diameter were not totally suppressed, the continuity of the larger vessels was often broken by the intrusion of giant cells into the vascular system. It seems likely therefore, that their efficiency as conducting elements was impaired.

Population density and soil moisture had a significant effect ($p < 0.01$) on root resistance to water flow. Resistance increased with population density of nematodes and with decreasing soil moisture content (Fig. 2).

The diffusive resistance of tomato plants infected with *M. javanica* increased as infection progressed and was always higher than in uninfected plants (Fig. 3). Regression coefficients were significantly different ($p < 0.05$) between the infected and non-infected plants. The resistances of leaves obtained from non-infected plants were lower than those from infected plants at any given time after inoculation. The infected plants did not show any significant stunting or other foliar symptoms at the end of the experimental period.

Water potential of leaves from infected and healthy plants over an extended period of 8 weeks after inoculation is shown in Fig. 4. The

1 regression coefficients were significantly different ($p < 0.05$). Water
2 potential in the infected plants decreased with time of inoculation at a
3 greater rate than that of the healthy plants. Inoculation of the tomato
4 plants with 6,000 larvae of *M. javanica* did not produce stunting or other
5 foliar symptoms.

6 Fig. 5 shows the relationship between leaf diffusive resistance and
7 water potential for both the infected and healthy plants. The leaf diffusive
8 resistance increased non-linearly as water potential decreased. Even though
9 the data were somewhat scattered, the regression line drawn indicates that
10 the diffusive resistance of leaves following inoculation was invariably
11 higher ($p < 0.05$) than the diffusive resistance of healthy plants at the same
12 water potential.

13 Transpiration rate of whole infected tomato plants over an extended
14 period of 8 weeks of inoculation did not differ significantly (Fig. 6) from
15 those of the healthy plants. The plants did not produce any significant
16 visual stunting, wilting or other foliar symptoms throughout the experimental
17 period.

18

19 ## Discussion

20 Histopathological studies indicated that disruption and abnormality of
21 xylem vessels occur when giant cells are formed in response to infection by
22 *M. javanica*. The results suggest that one consequence of this disruption
23 is a decrease in the rate of uptake of water by the roots and in the rate of
24 translocation. Studies on the interference of sap flow in the vascular
25 system of galled apple trees and tomato plants (8) support this hypothesis.

26 As a result of xylem disruption, root resistance to translocation of
27 water increased and water potential in the leaves decreased, although it
28 was not severe enough to cause wilting in any of the experiments. It is
29 possible therefore that the plant has some means of maintaining water

1 potential following infection by *M. javanica*. Uninfected healthy plants
2 maintain high turgor when water is limiting through closure of stomata
3 that effectively reduces transpiration (7) and it is likely that infected
4 plants have a similar regulatory response. However, the critical question
5 is, are infected plants more efficient at conserving water than uninfected
6 plants? The experimental results in this paper indicate that they are,
7 because at the same water potentials, infected plants have a higher stomatal
8 diffusivity than uninfected plants (Fig. 5). Transpiration measurements
9 on the other hand failed to indicate a statistically significant difference
10 between infected and uninfected plants (Fig. 6).

11 The mechanisms whereby water is conserved in the infected plant are
12 not understood although it is likely that the stimulus for the response
13 is hormonal. Thus, Itai and Vaadia (1971) have shown that moisture stress
14 is associated with a decrease in transport to the shoot system of cyto-
15 kinins synthesised in root tips. It is possible that xylem disruption
16 following infection by *M. javanica* not only reduces translocation of water
17 to shoots but hormones also, thereby eliciting stomatal closure. Experi-
18 mental data (in preparation) did in fact suggest that amounts of cytokinins
19 and gibberellin translocated from roots to shoots decreased and amounts of
20 abscissic acid increased in the leaves following infection.

21 It is also concluded that where wilting and stunting do occur in
22 infected plants, either the growth conditions are probably suboptimal
23 thereby inhibiting the plants ability to conserve water or the infection
24 level is so high that the plants regulatory mechanisms are insufficient to
25 cope with the disruption associated with infection.

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Figure 1 Tomato roots infected by *Meloidogyne javanica*.

A. Longitudinal section showing absence of secondary xylem in the region of giant cells.

B. A transverse section showing a cluster of giant cells surrounded by abnormal xylem.

a - nematode; b - giant cells; c - abnormal xylem vessels; d - normal xylem vessels.

A
d
c
b
a

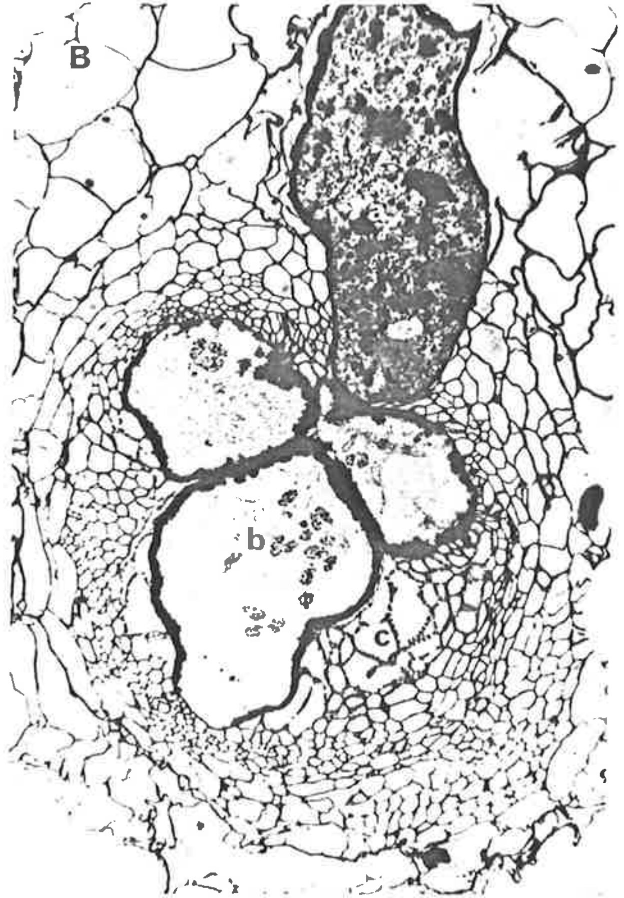
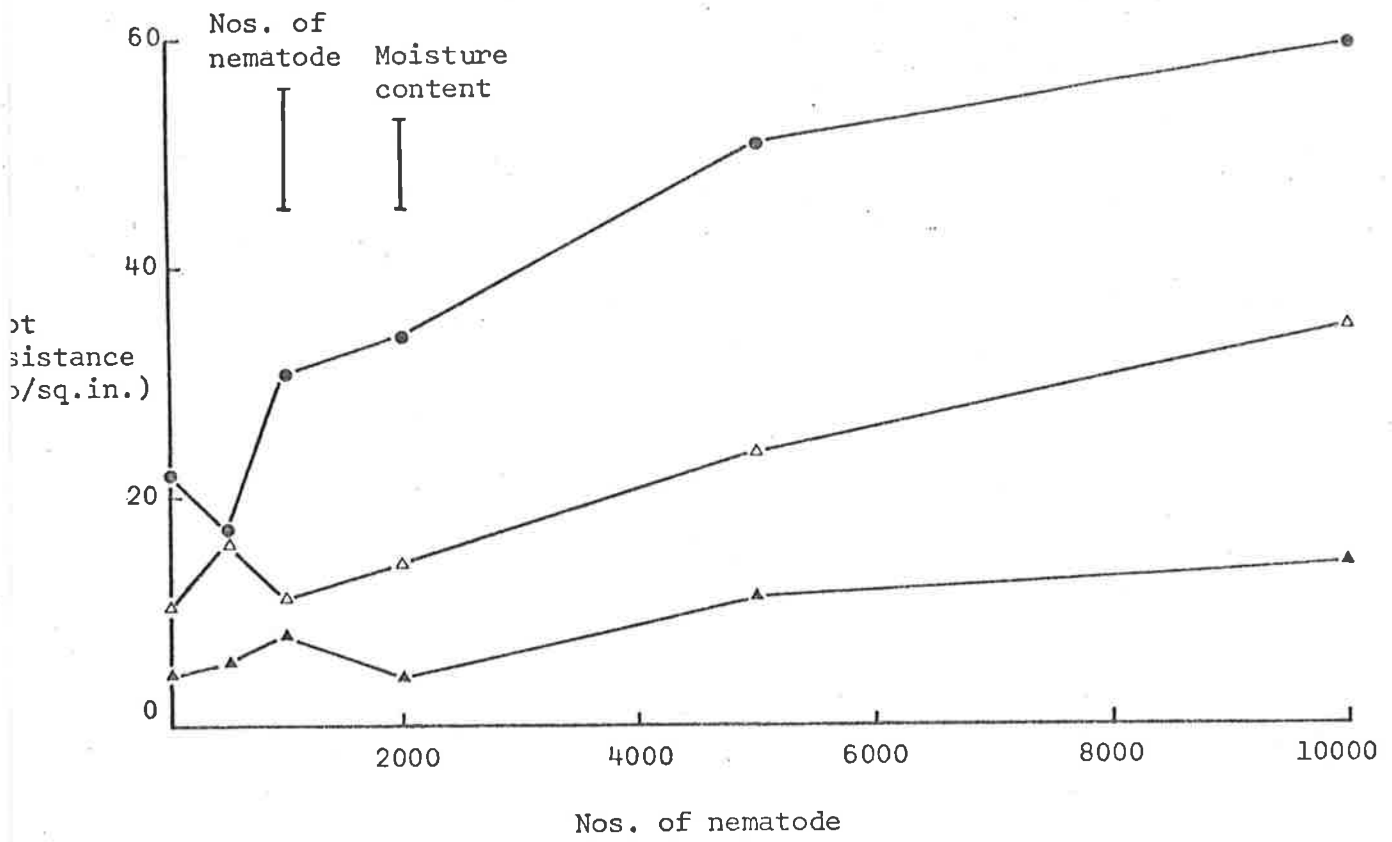
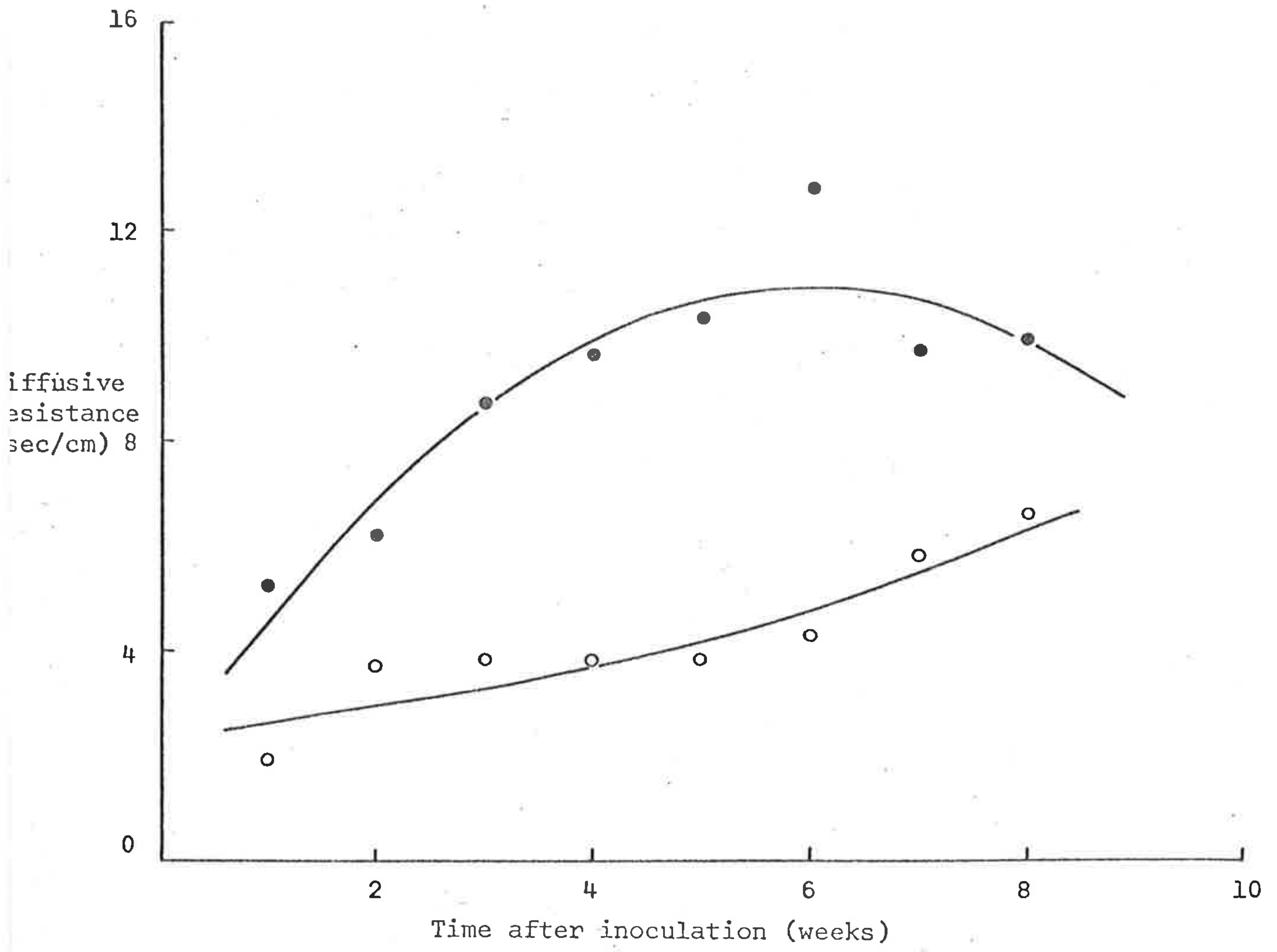

B
b
c

Figure 2 The influence of population density of *M. javanica* on the resistance of the roots of tomato plants to water flow at various soil moisture contents, forty days after inoculation. Soil moisture contents used were 8% (●), 16% (Δ) and 25% (▲) w/w. Each point is the mean of four replicates. Vertical lines indicate L.S.D. at $p < 0.01$.



Nos. of nematode
Moisture content
60
40
ot
sistance
o/sq.in.)
20
0
2000
4000
6000
8000
10000
Nos. of nematode

Figure 3 Regression lines showing the relationship between time and diffusive resistance of tomato plants with (•) and without (o) *M. javanica*.
Each point is the mean of 6 replicates.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sec/cm) 8
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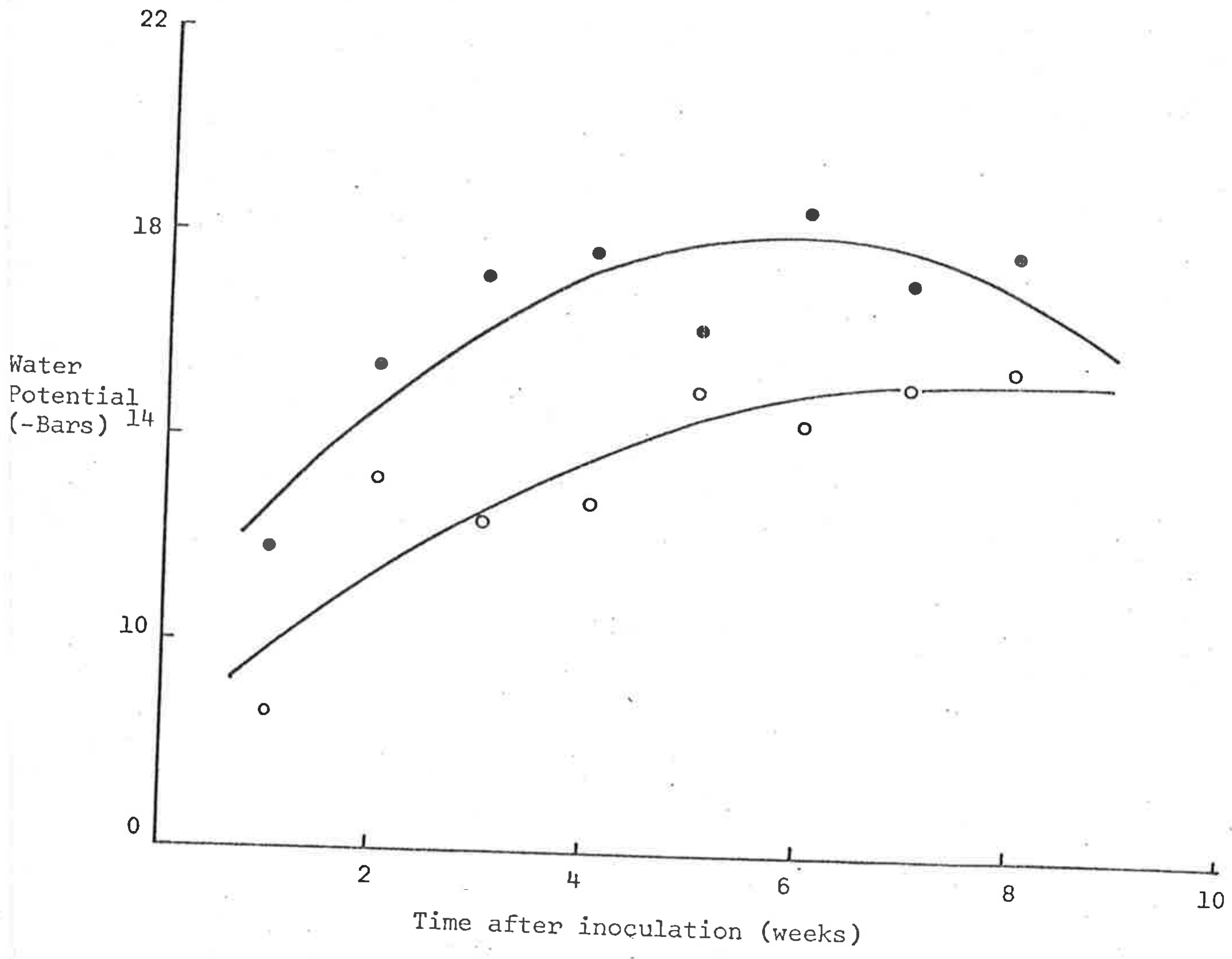
0
2
4
6
8
10
Time after inoculation (weeks)

Figure 4 Regression lines showing the relationship between time and water potential of tomato plants with (●) and without (o) M. javanica.
Each point is the mean of 6 replicates.



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18
Water
Potential
(-Bars)
14
10
0
2
4
6
8
10
Time after inoculation (weeks)

Figure 5 Regression lines showing the relationship between water potential and diffusive resistance of tomato plants with (•) and without (o) *M. javanica*.

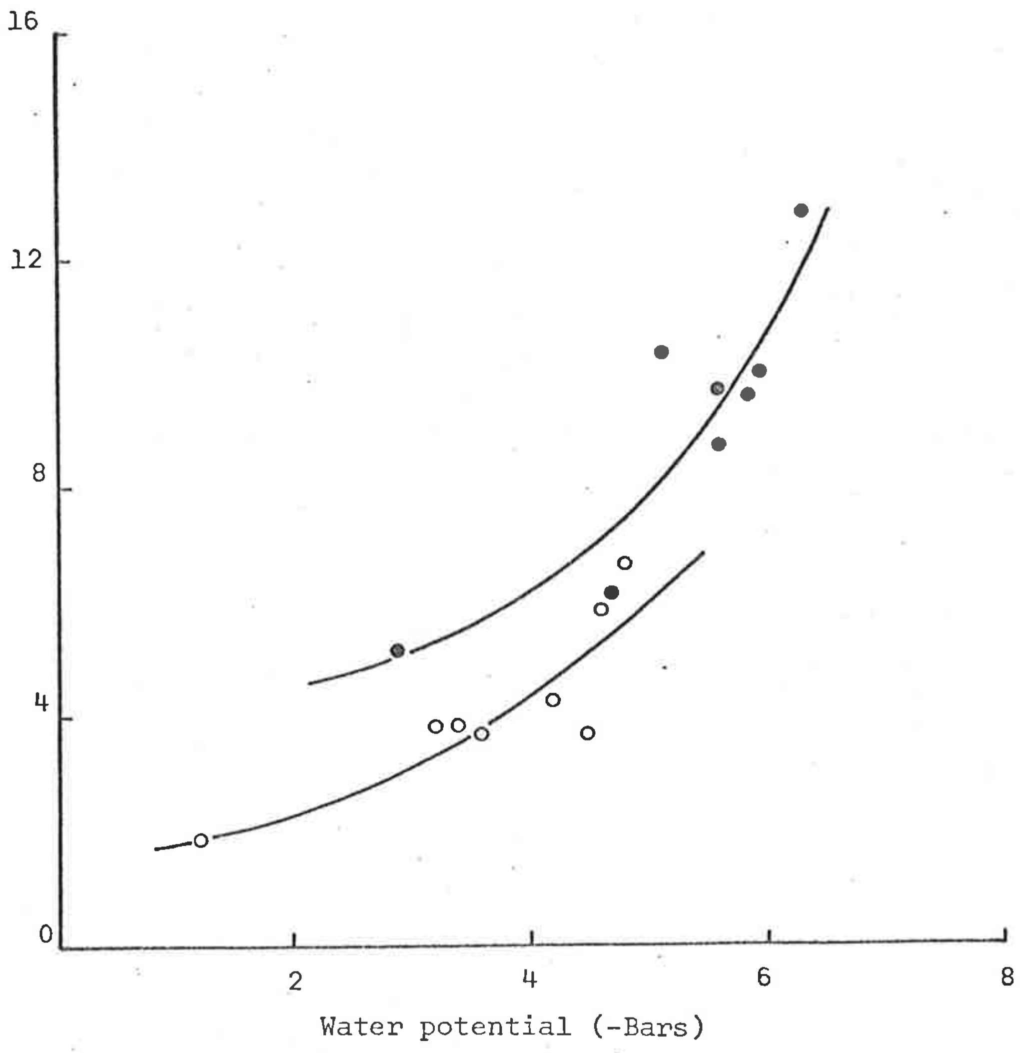
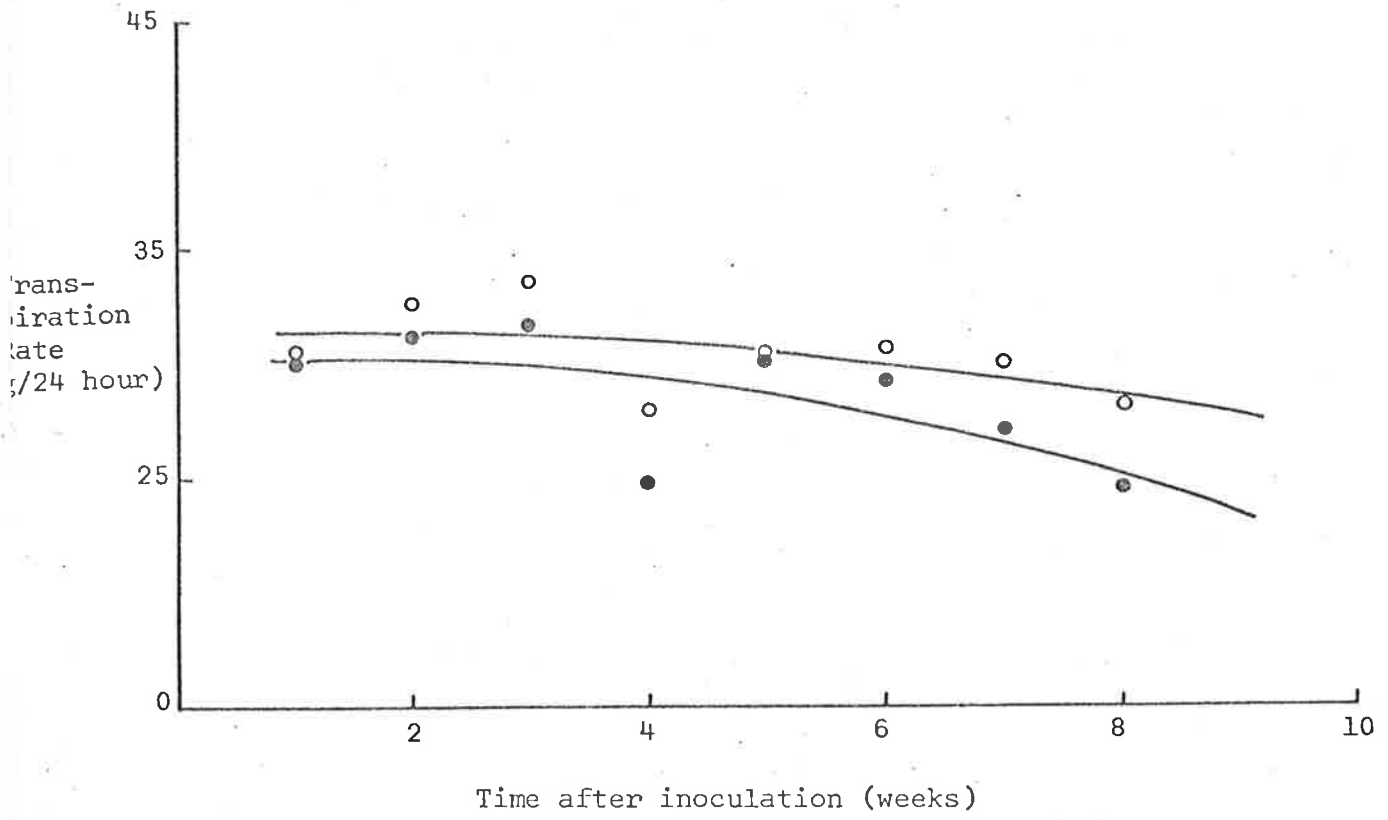

16
12
8
4
0
Diffusive
Resistance
(sec/cm)
2
4
6
8
Water potential (-Bars)

Figure 6 Regression lines showing the relationship between time and the whole-plant transpiration rate of tomato with (●) and without (o) *M. javanica*. Each point is the mean of 6 replicates.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ate
/24 hour)
2
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10
Time after inoculation (weeks)

Submitted for publication: Nematologica.

Sariah Meon[1)] The influence of infection by *Meloidogyne javanica* on growth hormones in tomato plants

Previous work on the influence of infection by *Meloidogyne javanica* (Treub) Chitwood on changes in free proline (Meon *et al.* a in press) and on the water relations (Meon *et al.* b in press) of tomato plants suggested that they were physiological processes that tended to maintain the health of the plant. Infected plants had a higher resistance to diffusion of water vapour from stomata than non-infected plants of the same water potentials. Thus the diseased plant conserved water and a possible mechanism whereby this could be achieved was by the activity of the plant's hormones. Accordingly, an attempt was made to study the changes in gibberellin, cytokinin and abscissic acid in root tissues, xylem exudate and the tops of tomato plants infected with *M. javanica*.

Materials and Methods

Tomato seedlings were inoculated with 7,000 larvae of *M. javanica* for five weeks after which the plants were cut off at the cotyledonary node, and the xylem exudate collected. Uninoculated plants were used as controls. Root tissues from uninfected plants and galls from infected plants were also collected. Gibberellins in the exudate and root tissues were extracted using the modified procedure of Goldschmidt and Monselise (1968). The air-dried chromatograms were sectioned and the activity was assayed

by the barley endosperm test (Coombe, Cohn and Paleg, 1967 a,b). Cytokinin was assayed by the soybean callus test (Miller, 1963).

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For determination of abscissic acid, samples of tops and roots were homogenised in an acetone:water mixture, and the acetone fraction separated by adding chloroform and centrifuging. The purified extract was strip loaded onto chromatogram paper with abscissic acid standards. Spots were eluted and methylated with p-tolysulphoryl methyl nitrosoamide until yellow and these solutions were then dried down under nitrogen and appropriate quantities of ethyl acetate were added prior to loading onto the G.L.C. (Model 409 Beckman gas chromatograph).

Results and Discussion

Assays for gibberellin failed to detect any statistically significant differences between the infected and uninfected plants, but less cytokinin was in infected than uninfected plants ($p < 0.05$, fig. 1). Abscissic acid concentrations increased in infected tomato plants (Table 1), the increase being more pronounced in the tops than in the roots.

Just as stunted growth may be associated in part with decreased cytokinin in the xylem exudate (Brueske and Bergeson, 1972) so might symptoms of stunting, wilting and loss in yield in infected plants be associated with hormonal changes. However, in previous experiments (Meon *et al.*, a and b, in press), tomato plants infected with *M. javanica* showed no signs of wilting or stunting in spite of reduced translocation of water to the tops caused by xylem disruption in the roots, probably because stomatal resistance to the diffusion of water vapour was greater in infected than in uninfected plants at the same water potentials. The increase in abscissic acid concentration in infected plants may be one of the mechanisms whereby water potential is maintained as this hormone is known to influence stomatal closure and reduce transpiration (Wright and Hiron, 1972; Wright, 1972; Hiron and Wright, 1973). That cytokinin concentration is lower in water-stressed plants (Itai and Vaadia, 1965) and that kinetin stimulates

transpiration (Livne and Vaadia, 1965) suggests that decreased amounts of cytokinins in the infected plants may also be associated with the conservation of water potential.

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Figure 1

Cytokinin activity extracted from healthy roots (o) L.S.D. ($p < 0.05$) = 63.04, galls (●) L.S.D. ($p < 0.05$) = 99.76, xylem exudate of healthy plants (Δ) L.S.D. ($p < 0.05$) = 109.73 and infected plants (▲) L.S.D. ($p < 0.05$) = 230.80 as measured by the soybean callus test.

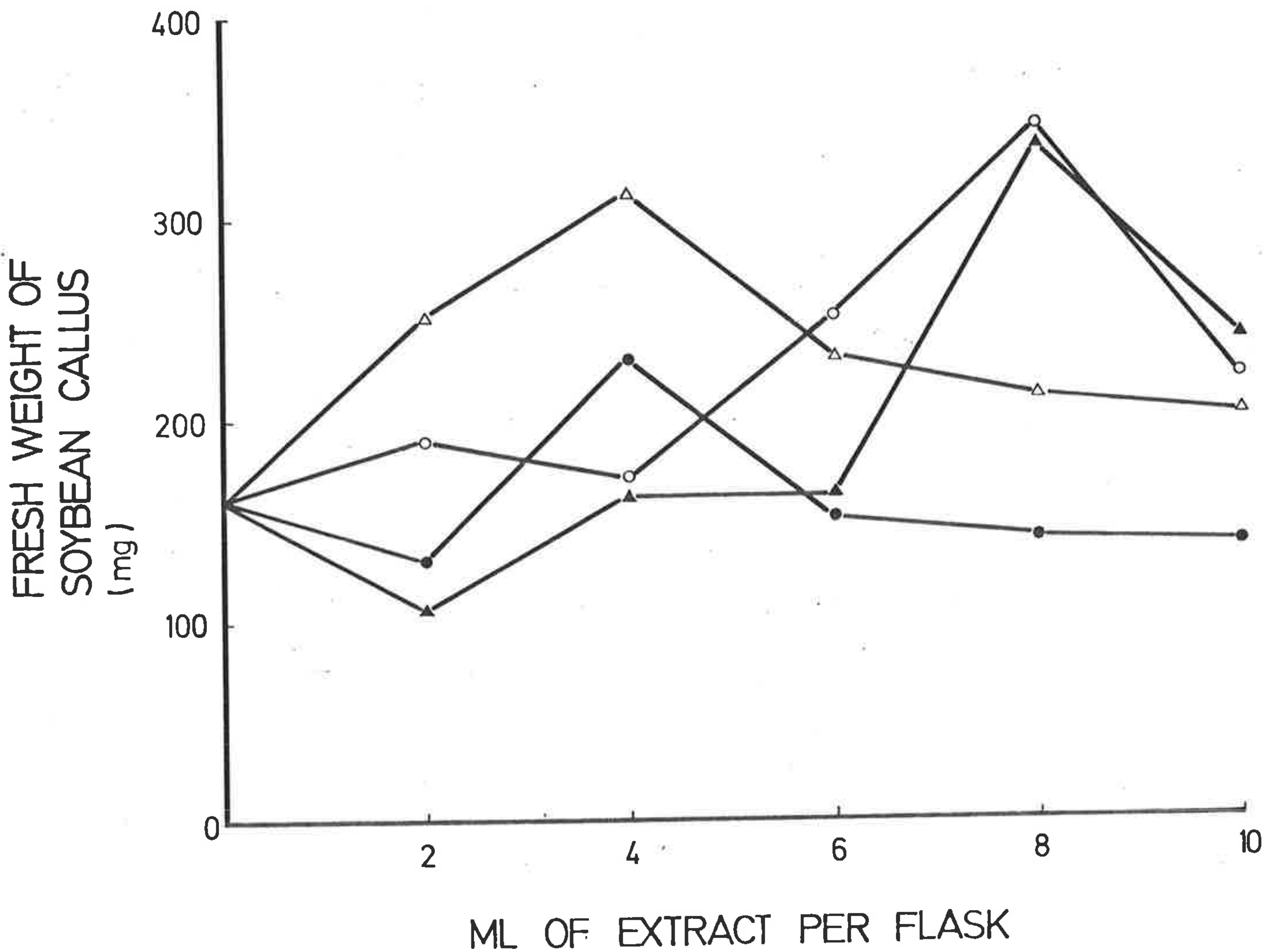

Table I

Concentration of abscissic acid (ng/mg fresh weight) in tops and roots of tomato plants with and without *M. javanica*. Each value is the mean of three replicates.

	Roots	Tops
Infected	7.10	26.23
Control	5.72	15.92
L.S.D. ($p < 0.05$)	1.20	6.09