THE INFLUENCE OF _MELOIDOGYNE INCognITA_ (NEMATODA, MELOIDOGYNIDAE) ON THE PHYSIOLOGY AND YIELD OF _PHASEOLUS VULGARIS_

by

HADDISH MELAKEBERHAN

M. Sc. (1980), Imperial College of Science and Technology, University of London, England
Diploma-Agriculture (1974), Ambo Institute of Agriculture, Ethiopia

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Approval

Name: Haddish Melakeberhan
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Examining Committee:
Chairman: Dr. K.K. Nair

Dr. J.M. Webster, Senior Supervisor

Dr. R.C. Brooke, Co-Senior Supervisor

Dr. G.R. Lister

Dr. W.E. Vidaver, Public Examiner

Dr. T. Vrain, Research Scientist, Agr. Cda., Public Examiner

Dr. D.R. Viglierechio, University of California, Department of Nematology, External Examiner

Date Approved: 11 March 1986

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The influence of Meloidogyne incognita (Nematoča, Meloidogynidae) on the Physiology and Yield of Phaseolus vulgaris

Author: [Signature]
Haddish Melakeberhan

(name)
March 11, 1986
ABSTRACT

The mechanisms by which root-knot nematodes decrease crop yield were studied using *Meloidogyne incognita* infections of *Phaseolus vulgaris* over 5 wk periods following host germination. Singly grown plants (from 3 to 13 day-old) were inoculated with from 0 up to 10,000 *M. incognita* second-stage larvae. The photosynthetic and respiration rates, chlorophyll content, inorganic (nutritional and structural) elemental concentration and content, and growth and yield components were measured at intervals from 0 to 4 wk after nematode inoculation.

When inoculated 3 d after germination, the photosynthetic rate (leaf area basis) decreased significantly with level of infection within 3 d. Plants one week or older at the time of inoculation did not show such a decrease on the basis of leaf area, chlorophyll, shoot nitrogen or shoot potassium concentration until a week after inoculation. In all instances, this rate decreased with the duration and level of infection. Chlorophyll content decreased 2 wk after inoculation. Respiration rate had no consistent relationship with either level or duration of infection. Plant weight and most elements, yield components and leaf area decreased significantly with increasing level of infection from 1, 2 and 3 wk after inoculation, respectively. However, the concentration of nutrient elements in the plant did not change until one week after inoculation at which time calcium tended to increase while potassium and zinc decreased.
The results suggested that the influence of the nematode on plant host physiology might be primarily by interfering with the uptake of nutrient elements and altering their quantity and distribution within the plant. This, in turn, leads to decreased photosynthesis and subsequently to loss of yield. Supplementary potassium (2-4X) resulted in an increase in the concentration of leaf potassium and an increase in the photosynthetic rate and yield components of nematode-infected plants compared with those receiving normal or no supplementary potassium. The location and concentration of potassium in the plant is likely a major factor in influencing the yield of nematode-infected crops.

This study identifies for the first time some of the possible mechanisms that lead to nematode-induced crop loss.
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CHAPTER I
GENERAL INTRODUCTION

Plant-parasitic nematodes include some of the most important plant pathogens that cause economic crop loss. Nematodes occur as endo- or ecto-parasites of roots and a few species parasitize stems and/or foliage (Slack, 1963; Webster, 1975). Most plant-parasitic nematodes are primary pathogens but some species act as vectors of other pathogens (Pitcher, 1965) or interact with other pathogenic micro-organisms resulting in disease complexes (Powell, 1971; Taylor, 1979). Although environmental and nutritional factors may modify disease expression (Dropkin, 1969; Webster, 1969), crops infected by nematodes may show a) patchy growth in the field, b) general reduction in growth, c) wilting, d) delayed or inhibited flowering, and e) lower fruit and fiber yields (Dropkin, 1980). The influence of plant-parasitic nematodes on these disorders and in other symptoms at the cellular, tissue and whole plant level has been extensively studied (see reviews by Seinhorst, 1961; Dropkin, 1969; Webster, 1975; Bird, 1974; Jones, 1981). However, the mechanisms by which nematodes induce yield loss and the precise estimation of this loss have received very little attention.
Dowler & Van Gundy (1984, p.8) summarized the situation:

"Our present estimates and determination of crop loss from any given factor leave much to be desired. Experiments to measure crop loss are difficult to design because of the many overlapping interactions involved. Nonetheless, it is necessary to do the best job we can in making such estimates and determinations".

In order to address this message, one has to identify the components that determine crop yield under optimal growth conditions, and ascertain how nematodes affect these components so as to decrease crop yield.

Crop yield is the result of a complex of interacting physiological processes and morphological characteristics that can be studied and quantified by considering the influence of a range of factors on these processes (Loomis & Adams, 1980). For optimum growth, a plant requires certain growth conditions both above and below the soil surface, so that its physiological processes can produce new biomass. One of the difficulties in examining the physiological processes is the complex nature of their interactions and the influence of these interacting factors on host physiology and growth (Schoeneweiss, 1978). The complexity of these physiological processes is further influenced by nematodes and other disease-causing agents (Fattah & Webster, 1983) whose effects vary with the species of host and parasite, population size, host type and the age and stage of infection (Bird, 1974; Barker & Olthof, 1976; Sidhu & Webster, 1981). Some of the more important factors that influence host-nematode interaction are reviewed below.
Despite its central role in plant growth and associated crop yield, photosynthesis has been almost overlooked in nematode-host interaction studies. Loveys & Bird (1973), Wallace (1974) and Franco (1980) are the only significant reports of such studies and they showed decreased rates of photosynthesis in crop plants infected with nematodes. Reasons for the decreased photosynthesis include (1) a reduced supply of root-derived factors (the nature of which are, as yet, unknown) (Loveys & Bird, 1973) and (2) deformation of vascular tissue by nematodes in which translocation of nutrient elements to the shoot is believed to be more important than photosynthate translocation to the roots (Wallace, 1974). This latter point was disputed by Bird & Loveys (1975), McClure (1978) and Meon et al. (1978).

Studies on the uptake of water as affected by nematodes generally indicate that nematode injury to the root system decreases both water and solute absorption and their translocation to the shoots. Different inoculum levels of Meloidogyne incognita significantly decrease the water absorption capacity of tomatoes compared with that of control plants (Alam et al., 1975; Alam & Saxena, 1975 a). Similar results have been reported for castor and tomato plants infected with Rotylenchulus reniformis (Islam & Alam, 1975), for cauliflower and cabbage infected with Tylenchorhynchus brassicae (Alam & Saxena, 1975 b) and for tomato infected with M. javanica (Meon et al., 1978). The influence of nematode-induced modified
water uptake on other physiological processes was shown by Evans et al. (1977) who suggested that the increased calcium uptake per unit of water transpired in nematode infected plants may be an indicator of direct or indirect nematode damage.

When nematodes infect plants, nutrient levels may be decreased, selectively increased and/or their distribution between the different plant parts altered which results in a change in host physiology. Oteifa (1952) reported a decrease in percent root nitrogen, phosphorus, calcium, manganese and potassium in lima bean while Hunter (1958), using tomatoes, found increased concentrations of nitrogen, phosphorus, potassium and magnesium in the roots of *M. incognita* infected plants compared with the controls. Maung & Jenkins (1959) and Shafiee & Jenkins (1963) showed increases in the concentration of nitrogen, phosphorus, potassium and sodium in pepper roots infected with *M. incognita acrita* but not in the shoots. Bergeson (1966), using a split root technique and *M. incognita* infected tomato plants, showed that more nitrogen, phosphorus and potassium occurred in galled tissues than into non-galled tissues. Trudgill et al. (1975), however, showed a decrease in percent nitrogen, phosphorus, potassium and magnesium in potato roots infected with *Globodera rostochiensis*. Recent studies by Nasr et al. (1980) have shown increased concentration of calcium, copper and iron in the shoot and potassium in the roots of *M. incognita* infected almond and peach root-stocks. Similar results have been reported for calcium in potatoes infected with
Globodera rostochiensis (Evans et al., 1977; Evans, 1982) and in barley infected with Heterodera avenae (Price et al., 1982; Price & Sanderson, 1984).

In a plant-nematode interaction, particularly with specialised parasites like root-knot nematodes, the role of plant growth regulators in determining host response to infection is important (Sawhney & Webster, 1975). However, their presence, source and distribution in the infected roots remains unclear. Indoleacetic acid (IAA) type growth regulating substances have been found in extracts of nematode-induced galls and in most cases the nematodes have been implicated (Bird, 1962; Viglierchio & Yu, 1968; Dropkin; 1969). However, Dropkin (1980) believed that there is little evidence to show that nematode-produced IAA-type substances caused the galling. Setty & Wheeler (1968) suggested that root-knot nematodes either introduce these growth regulating substances themselves or stimulate the host to produce quantities sufficient to increase their concentration in the infected roots. Although Bruske & Bergeson (1972) disagreed, there are several reports that showed quantitatively more growth regulating substances being located in nematode-induced galls than in the adjacent tissues of uninfected roots (Balasubramanian & Rangaswani 1962; Bird, 1962; Setty & Wheeler, 1968). These conflicting observations may be due to differences in assay technique, to experimental design or to the specific host and parasite species and the stage of infection (Bird et al., 1980). Nonetheless, it seems likely that
In some ways the parasite nematode influences the role of plant growth hormones and this is associated with modification of growth patterns.

In order to find ways of preventing loss of yield it is essential to know first what nematode parasites do to a plant host (Webster, 1980). This requires a clear understanding of the influence of nematodes on those physiological processes affecting yield. However, there is no report of yield loss estimation involving nematode-infected plants in the literature that is (1) physiologically based or (2) does not include multiple generations of the experimental nematode. Multiple generations of the experimental nematode, together with other interacting factors (e.g., fungi, soil conditions), complicate the interpretation of results and so limit our understanding of the physiological processes in the host because they affect the precise quantification of loss of yield (Main, 1983).

Therefore, the objective of my thesis is to precisely quantify yield loss and determine some of the physiological mechanisms by which a nematode species induces this loss. The first step was to select an economically important parasitic nematode, and a host plant that produces seed within a single generation of the nematode. Based on these considerations, the root-knot nematode (*Meloidogyne incognita*) and a dwarf French bean host (*Phaseolus vulgaris*) were selected as the parasite-host system for experimental purposes.
The genus *Meloidogyne* is one of the five major crop pests worldwide (Sasser, 1977). *Meloidogyne incognita* is highly polyphagous and is one of the most specialised, obligate, plant-parasitic nematodes (Dropkin, 1980). Lamberti & Taylor (1979) and Sasser & Carter (1984) provide a detailed analysis of the taxonomy, physiology, anatomy, host range and biology of this species (genus). Only a brief description of the life cycle and associated host response is provided here.

Like any other plant-parasitic nematode, *M. incognita* has four larval stages and one adult stage and under favourable conditions, the life cycle takes 4 - 5 wk. Eggs are laid in a gelatinous matrix and may be found adhering to the roots of the host or free in the soil. The first larval molt occurs within the egg shell and the second stage larvae hatch and invade plants usually just behind the root cap in the region of intense meristematic activity (De Guiran & Ritter, 1979). The larvae penetrate the cortex and establish themselves within the vascular tissues where they induce giant cell formation and root galling (Paulson & Webster, 1970; Bird, 1979).

*Meloidogyne*-induced giant cells are multinucleate, contain dense cytoplasm and have highly invaginated, thickened cell walls. The larvae feed on the giant cells, start to swell and, after the second molt, remain enclosed and non-feeding until the final moult to maturation. The metabolic activity of the giant cells, however, increases with the age of the nematode until the end of egg laying, and declines thereafter. As a result of giant
cell and gall formation, the normal differentiation of the host vascular tissues is disrupted (Paulson & Webster, 1970; Bird, 1979) and biochemical changes occur that subsequently alter the host physiology (Setty & Wheeler, 1968; Endo, 1971; Dropkin, 1980). Thus, metabolite distribution in the roots is upset and galled plants usually transfer large quantities of substances, such as sugars and amino acids (Bird, 1966; Singh et al., 1979; Basu & Sukul, 1983) to the rapidly growing nematode via the giant cells.

The experimental host, P. vulgaris, is an economically important crop and its physiology is relatively well known (e.g. Raafat et al., 1968; Makrides & Goldwhite, 1980; Jenkins et al., 1981 a & b). It is an important source of protein and an enricher of soil nitrogen which has significant effects on other crop production.

After selecting the experimental parasite and host systems and in order to reach the objective, I asked the following questions:

(1) Which of the major morphological characteristics and physiological processes of P. vulgaris are changed by a M. incognita infection?

(2) Are there specific relationships between host development and function and the nematodes' development and intensity of infection?

(3) What are the possible mechanisms by which the nematode induces these changes?
A *Phaseolus vulgaris* L. cultivar, that produces seed within 5 wk of germination, was the host used for all experiments. In selecting the cultivar, the following three criteria were considered: (1) The host cultivar had to be susceptible to the root-knot nematode, *M. incognita*, (2) readily grown under controlled environmental conditions and (3) had to be of compact size. The size was important for two reasons: (a) it was decided to use the whole plant, as opposed to the single leaf procedure, for CO₂ exchange measurements because a leaf may not be as representative of the plant's behaviour and it may be lost due to mechanical damage, (b) the selected host has a short life cycle and completes ontogeny in a relatively short period (Sestak, 1977 b) and the nematode undergoes a major morphological and physiological change and mature's in 4 wk. Consequently, appropriately replicated samples can be taken providing that the CO₂ exchange rate measurements are done in a timely manner so that conflicting interpretation is minimized.
These two factors and technical problems of handling big chambers in the laboratory made it necessary that the plant be compact at maturity so that it fits into relatively small plant chambers (see later).

Hence, four dwarf French bean varieties, Topnotch Golden Wax (TGW), Kentucky Wonder Wax (KWW), Tender-Green (TG) and Roma Bush (RB) were obtained from a commercial supplier (Buckerfields) and screened for percent germination. About 20 seeds from each variety were sown singly in 9 x 7.5 cm diameter plastic pots containing potting soil. The pots were maintained in a greenhouse at 22 ± 5 °C with a 16 hr photoperiod supplemented by cool-white fluorescent lamps. The pots were watered daily and the seeds assessed for percent germination one week later. TGW and KWW had the highest percent germination and were selected for further screening of soil type for best growth, growth pattern and susceptibility to M. incognita. Twelve seedlings from each of these cultivars were germinated individually in potting soil and 1:1 sand:silt mixture and kept under the growing conditions described above. Growth pattern, height and width of the plants were measured weekly so that appropriate chamber size could be designed for the CO₂ exchange rate measurements. Plants were watered weekly with Miracle-Grower 15 - 30 - 15 commercial fertilizer mixture (Sterns' Nurseries Inc., N. Y., USA) and tap water depending on the soil moisture in the top soil of the pots. Both cultivars grew best in the loam soil with KWW being larger than TGW after
4 wk. Six seedlings of each cultivar growing in the loam soil were inoculated with approximately 1000 freshly hatched *M. incognita* larvae. Two weeks later, roots were washed and an assessment of gall formation was made. There was no apparent difference in galling between the two cultivars. However, because TGW produced a smaller plant than KWW at maturity, it was selected as the experimental host cultivar.

**PLANT GROWTH CONDITIONS**

Temperature and light intensity are among the primary factors that affect plant growth. *Phaseolus vulgaris* cultivars may respond differently to the conditions to which they are subjected. Nonetheless, the general developmental trend of photosynthetic and dark respiration rates and chlorophyll content are likely to be similar. The period of maximum foliar chlorophyll content and apparent (net) photosynthetic (APS) rate occurs when the primary leaves are fully expanded and declines thereafter with a slight increase at the time of flowering and pod formation stages (Sestak, 1977a & b; Sestak, *et al.*, 1977). For example, healthy bean plants were grown in loam soil at 70 % R. H., 23 °C, 14 hr photoperiod at 1350 ft-c (Fraser & Bidwell, 1974) and at 80 % R. H., 245 Jm⁻².s⁻¹ and under natural conditions (Louwerse & Zweerde, 1977). At the fully expanded
stage of the primary leaves, a maximum APS rate of 20-30 mg CO$_2$.dm$^{-2}$.hr$^{-1}$ was recorded in these *P. vulgaris* plants (see eg. Fraser & Bidwell, 1974; Louwerse & Zweerde, 1977). This rate is within the 20-35 mg CO$_2$.dm$^{-2}$.hr$^{-1}$ range of herbaceous Calvin cycle crops (Sestak *et al.*, 1971).

Dark respiration rates of bean plants are highest at the early seedling stage (Opik, 1966), 2 - 3 d after germination.

Most dwarf (bush) bean cultivars are short day (14 hr day and 10 hr night cycle) plants (Purseglove, 1972). Although in the preliminary tests plants grew best in loam soil supplemented with a Miracle-Grower 15 - 30 - 15 commercial fertilizer, it was necessary that plants for experiments receive a balanced nutrient supply. Consequently, a standard Hoagland's (1939) solution was utilized. Thus, a series of tests were conducted in a Conviron growth chamber to determine the optimum temperature, light intensity and the frequency of fertilizing the TGW cultivar with full strength Hoagland's solution. Plants grew best when they received Hoagland's solution or tap water on alternate days. One might expect an increase in salt concentration in this type of soil with the additional nutrient application. However, no evidence of increased salt concentrations in the soil was observed and no salt-related symptoms were evident in the plants. The best growth conditions were at 21 °C, 14 hr day at quantum flux density (400-700 nm) of 400 μE.m$^{-2}$.s$^{-1}$ and 16 °C 10 hr night cycle. Light was provided by six incandescent (60 W, Westinghouse) and eight, cool white
fluorescent (F72T12 CW-1500, G. M.) lamps located at 90-110 cm above leaf canopy. Once the appropriate growth conditions had been determined, all further experiments were conducted under these standardised conditions.

Seeds were germinated in sterilized 1:1 sand:silt mixture in a Conviron growth chamber. At 2–3 d after germination, seedlings were selected for uniformity of shoot growth and transplanted singly into 9 x 7.5 cm round plastic pots containing similar soil. Experimental plants were arranged in a completely randomised design and maintained under the day and night conditions described above. Plants were watered with full strength Hoagland’s solution or tap water on alternate days for each series of experiments.

NEMATODES

1 Stock Culture Maintenance

*Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 were provided by Dr. Farkad Fattah, former fellow graduate student, and maintained in either lima bean (*P. lunatus* L. cv. L-136) or tomato (*Lycopersicon esculentum* L. cv. Bonny Best). These plants were infected with nematodes by inoculating the
soil with either infected root material or hatched larvae (see below). Plants were watered as required (depending on the moisture content of the top of the soil in the pots) with tap water to maintain the soil in a moist state and fertilized biweekly with either complete Hoagland solution or Miracle-Grower 15 - 30 - 15 fertilizer and maintained in a greenhouse at 26 ± 5 °C with supplementary light from cool-white fluorescent lamps to maintain a 16 hr day photoperiod.

2 Extraction and Inoculation

Egg masses were collected from infected bean and/or tomato plants after removing the shoots and carefully washing the roots in cold tap water. Egg masses were placed onto 4 cm diameter 90 mesh nylon sieves on glass Petri dishes with distilled water. In order to improve aeration, the level of the water in the Petri dishes was adjusted so as to completely cover the sieve mesh but not to completely cover the egg masses. The required number of Petri dish/sieve extraction units were placed in trays, covered with aluminium foil and the eggs hatched at 25 ± 5 °C in incubators and/or the laboratory.

Hatched larvae were collected daily by decanting them in suspension from the Petri dishes. After thoroughly mixing the larval suspension by blowing air through pipettes, larval
numbers were estimated by taking a 1 ml aliquot of the suspension and counting them on a gridded Petri dish using a dissecting microscope. The number of nematodes estimate was based on an average of three replicates. The required number of larvae were inoculated into the soil by pipetting the thoroughly mixed larval suspension into 3 - 5 holes in the soil around the plant stem (Wallace, 1974). Distilled water was inoculated into similar holes around the stems of control plants. If not required immediately, the larvae were stored at approximately 10 °C for up to 48 hr until needed for inoculation. Hatched larvae more than 48 hr old were discarded, and depending upon the numbers required, inoculations were done over a 24 hr period.

3 Nematode Recovery

As the physiological and morphological responses of the host plant are influenced by the degree of nematode infection (Wallace, 1974), where possible, it was essential to know as precisely as possible the number of nematodes infecting the plant. This, however, was not possible in all cases. Depending on the experiment, the data are expressed either on the inoculum level or on the number of nematodes recovered from the roots. At each sampling date or at the end of each experiment, plant roots were gently washed free of soil and 0.5 g fresh weight samples
of root were taken randomly from each plant. These samples were tied in cheese cloth, plunged into boiling 0.01% cotton blue lactophenol for 3-5 min (Hooper, 1970) and washed in cold tap water for 2 min. Samples were cleared in lactophenol and the number and/or developmental stages of nematodes in the roots were assessed microscopically.

\textbf{RHIZOBIUM CULTURING AND INOCULATION}

\textit{Rhizobium phaseoli} isolate RCC 324 (obtained from Ms Andrea Smulders, Department of Plant Sciences, University of British Columbia) were maintained in yeast extract mannitol agar (YEMA) medium composed of mannitol 10 g, plain agar 15 g, K$_2$HPO$_4$ 0.5 g, MgSO$_4$ . H$_2$O 0.2 g, NaCl 0.1 g, CaCl$_2$ 0.05 g, yeast extract 0.4 g, in 1000 ml distilled water at pH 7. At given intervals, a suspension of bacterial cells was prepared on a lamina flow bench in sterile distilled water and inoculations done by either the flamed loop technique into slants or by pipetting small droplets into 6 or 10 cm diameter disposable plastic Petri dishes containing the media. To minimize microbial contamination and desiccation, slants and Petri dishes were covered with either Para-Film or Saran Wrap and stored under laboratory conditions.
When required for use, bacterial suspensions were prepared from 3 - 4 week-old cultures and populations estimated using a bright-line (Spencer) haemocytometer. An estimated $2 - 2.5 \times 10^7$ bacterial cells were pipetted into the soil of each bean plant at transplanting time.

In the initial growth experiments, nodulation of bean roots was not satisfactory and, hence, a complete normal strength Hoagland solution was applied together with *Rhizobium* to minimize the chances of a nitrogen deficiency. Consequently, nodulation was not observed.

**EXPERIMENTAL DESIGN AND GROWTH MEASUREMENTS**

After determining the appropriate growth conditions ($21 \, ^\circ \, C$, 14 hr day; $16 \, ^\circ \, C$, 10 hr night cycle) of the host, tests were done to determine how long the nematode takes to reach the egg laying stage so that the duration of the experiments could be determined. It took 4 wk to complete the life cycle (Fig. 1) and no experiment was allowed to go beyond this period after inoculation.

Depending upon the experiment, from 3 d (before primary leaf expansion) to 13 (at the flower bud stage) day-old plants were inoculated with 0 (control) to 10000 larvae/plant and studied for up to 4 wk (Fig. 1). Each experiment had 4 or 5
replications and potted plants were arranged in a completely
randomised design in the growth chamber.

Destructive and non-destructive assay methods were used and
depending on the design of the experiment, leaf area, dry weight
and buds, flowers, pods and seeds (yield components) were
determined at intervals from zero time (just before inoculation)
to 4 wk after nematode inoculation.
The relationship between the developmental stages of the host, from seed germination to maturity, and the nematode, from inoculation (L2) to egg laying stage.

The nematode developmental stages were monitored microscopically by dissecting the roots at 1, 3, 8, 15, 22 and 27 d after inoculation. Nematodes were observed penetrating the roots up to 8 d after inoculation but not at 15 d. Since all experiments were conducted under similar conditions, it is assumed that the host invasion, increase in body size and moulting for the non-destructive assay experiments fall within the range of lines indicated in this diagram. However, it is possible that 1) nematodes may invade younger plants faster than they do older plants and 2) there may be overlapping stages of development of the larvae before the last moult, but it is unlikely that these developmental stages would have been significantly outside of the ranges in this diagram.
Leaf area as used here refers to single surface, and was determined by tracing leaves on 183 type infrared transparency films and their area determined either gravimetrically (Sestak et al., 1971) or using a Polar compensating planimeter. Dry weights of shoots (leaves and stems separately) and roots were determined after samples were dried in an oven at 65 - 70 °C for 3 - 4 d.

CO₂ EXCHANGE RATE MEASUREMENTS

The short life cycle and associated ontogenetic processes of the selected host and parasite required frequent measurements of CO₂ exchange rate. As it was not possible to measure the CO₂ exchange rate of more than one plant at a time using a closed system of measurement, an improved open system was designed.

1 Equipment

The components of the CO₂ exchange measuring and control system are shown in Fig. 2. Five cylindrical copper chambers with plexiglas tops were used, one as a reference chamber and four for the test plants. Each chamber had a volume of
approximately 3.4 l, a fan to mix chamber air, had adjustable temperature control and to improve light reflection was painted white on the inside surface. Air temperature within each chamber was measured using copper-constantan thermocouples and controlled to ± 1 °C by circulating cold water from a Lauda K-2/R refrigerated water circulator (Brinkmann Instruments) through water jackets around each chamber (Fig. 2). Air flow rate and light intensity to the chambers were monitored and could be varied.

The chambers containing the test plants were arranged beneath the lights on an adjustable platform. Four 500 W Dicrolite quartz iodide lamps equipped with IR reflecting mirrors were used as the light source for photosynthetic measurements. A saturating quantum flux density (400-700 nm) of 750 μE . m⁻² . s⁻¹, measured with an LI-190S quantum sensor (LI-COR, Inc.), was achieved by adjusting the level of the platform with two jacks. The IR component and heat from the lamps was minimized by a 9 cm deep waterbath of slowly circulating, cold tap water placed between the lamps and plant chambers.

An excess of outdoor (ambient) air (325-355 μl. l CO₂) was pumped by two Dyna pumps into a ballast flask (58.5 l) to minimize sudden ambient outdoor CO₂ fluctuations from reaching the plants. Ambient air was then pumped into each chamber continuously by separate H & B Wisa and Dyna pumps at a precisely regulated flow rate. Air from the chambers was passed
Figure 2

Diagram of the improved open system for CO₂ exchange rate measurement in which the experimental plants (PC) are in sealed chambers arranged with the reference chamber (RC) on an adjustable platform below the light source. The numbers in brackets refer to the number of items.
LEGEND

- AIR FLOW
- WATER FLOW FOR TEMPERATURE CONTROL
- MAGNETIC VALVES CONTROL
- TEMPERATURE DATA COLLECTION
- CO₂ EXCHANGE DATA COLLECTION

FLOW RATE ADJUSTED TO 300 ml/min

Diagram:
- Outdoor Air
- Filter
- Ballast Flask
- Pumps (2)
- Pumps (5)
- Flow Rate Control Valves (5)
- Dicrolite Quartz Iodide Lamps (4)
- Heat Filter (H₂O)
- Refrigerated Water Circulator
- Recorder
- IR Gas Analyzer
- Flow Meters (2)
- Solenoid (magnetic) Valves (4)
- PC Platform
- PC Preconditioning Bench
- Adjustable Platform
- PC
- PC
- Computer for Control and Data Acquisition
- RC
- Preconditioning Computer for Control and Data Collection
through two-way exhaust solenoid valves controlled for overlap, sample sequence and duration by an ES-130 computer (Digital Equipment Corp.). Sequential sub-samples of the air stream from the plant chambers and a continuous sub-sample from the reference chamber (flow rate 300 ml.min⁻¹) were analyzed differentially for CO₂ by an ADC-225 MK3 IR gas analyzer (The Analytical Development Co. Ltd, Hoddesdon, U. K.). The remaining air stream was exhausted through #604 flow meters (Matheson Co. Inc.) to the room (Fig. 2). When plant chambers were not being sampled, they received a continual supply of air which was exhausted into the room through the solenoid valves. All air and water flow connections were made with tygon tubing (Fisher Scientific Co.). Air lines were measured and tested at the flow rates used to ensure the same time constant between each chamber and the IR gas analyzer.

The CO₂ exchange data was recorded for continuous visual monitoring by a Moseley 7100B strip chart recorder and the computer was programmed to collect the CO₂ exchange and temperature data at set time intervals (see below) in sequence from each plant chamber, and to calculate the CO₂ exchange rates. Temperature and CO₂ calibration curves were programmed into the computer and checked at least at the beginning and end of each sampling day.
Testing the CO₂ Exchange Measuring System

Before using the open system for an extensive sequence of experiments, the following tests were done.

i) Flow rate determination

To ensure that CO₂ concentration would not limit the APS rate and to maintain a small differential in CO₂ concentration between incoming and outgoing air to the plant chambers, APS rates of one, two and three week-old *P. vulgaris* plants were measured at flow rates from 1 to 5 l.min⁻¹. For the chamber size and amount of plant material sampled, a flow rate of 3 l.min⁻¹ or more gave a quick, stable differential in CO₂ concentration.

ii) Determination of time constants

Since there was only one measuring sample air line between the solenoid valves and the analyzer, it was necessary to determine the time required for air to move from each chamber outlet to the analyzer so that air sampled for CO₂ exchange would not be mixed from chambers sequentially sampled. Empty, sealed chambers were provided with ambient air at the desired flow rate and, concurrently, a high concentration of CO₂ was quickly injected into the air stream at the outlet of each chamber. As it took 22 to 38 s to remove this high CO₂ concentration from the analyzer, no CO₂ data was taken from a plant chamber for 90 s after being placed in the measuring system.
iii) Diurnal pattern of photosynthetic rate

To determine the duration of more or less stable APS rates during a day, and thereby avoid difficulty in comparing plants which may have large diurnal fluctuation in APS rate (such as a bimodal pattern or mid-day depression), the APS rate of three to four 10, 14, 19, 23, and 26 day-old *P. vulgaris* plants was determined from dawn to dusk (14 hr). These ages were selected, in part, to correspond with sampling dates of future experiments (see below). It was found that the rates of photosynthesis were quite stable for 10 to 12 hr beginning about 2 hr after dawn (Fig. 3).

3 Experimental Procedure

Shortly after dawn, a batch of eight plants (2 replicates x 4 treatments) was preconditioned for about 2 hr in the laboratory to the light conditions used for APS measurements before being placed in the plant chambers. For the APS and dark respiration rate measurements, the entire shoot of each plant was sealed into the chamber. However, due to the small size and delicate stem of 3 day-old plants, the whole plant in its uncovered pot was enclosed in the sealed chamber in each instance. Although one might expect slightly higher dark
Figure 3
Diurnal patterns of mean hourly APS rates for *Phaseolus vulgaris* plants of different ages. (SE=standard error).
respiration and a slightly lower APS rates in the 3 day-old plants measured this way, repeated tests showed no significant difference in the CO$_2$ exchange rate of same-age plants in pots sealed in the chambers compared with those having only the shoot sealed in the chambers.

Based on the flow rate and time constants determined above, each plant chamber was sampled in sequence for 5 min. Within this 5 min interval, the computer was programmed to sample CO$_2$ exchange and temperature data seven times at 30 s intervals beginning 90 s after each chamber was inserted in the measuring circuit and stopping 30 s before the chamber was out of the circuit (see appendix I). At least three APS and two dark respiration measurements were taken, each for 5 min, of each replicate. This programme, with concurrent measurement of plants from each treatment forming a series, allowed for completion of the required APS and respiration measurements in less than 2 hr for each replicated series. As soon as the APS measurements for a chamber were completed the chamber was darkened for the respiration measurements. Each chamber was in darkness for 15 min before respiration was recorded and, when completed, the plants were replaced by a second group of four plants.

Using this sampling protocol, APS and dark respiration rate measurements of some 16 - 20 plants per day were taken. Chamber temperatures and air flow rates for the duration of these measurements was maintained at 22 ± 1 °C and 4 - 4.5 l .min$^{-1}$ respectively.
CHLOROPHYLL ANALYSIS

Chlorophyll content was measured by taking 5 mm diameter leaf discs from each plant. The discs were wrapped in black plastic to prevent photo-oxidation of pigments and stored frozen until extraction (Linder, 1974). Chlorophyll a and b were extracted in 100 % acetone and readings taken at 662 and 644 nm, respectively, on a Beckman model 35 spectrophotometer. Chlorophyll contents were calculated using the formulae of Arnon (1949) and Holm (1954) and expressed on a leaf fresh weight or area basis. See appendix II for more details of sample storage, extraction and chlorophyll reading procedures.

ELEMENTAL ANALYSIS

1 C-H-N Analysis

Dried shoots and roots were ground separately and 1.5 - 2.0 mg of the powder was used for C - H - N analysis in a Carlo Erba elemental C - H - N analyzer model 1106.
The concentration of potassium, calcium, manganese, iron, copper and zinc of ground and/or pulverized powder of plant tissues (leaves, stems, roots) was determined using X-ray energy spectroscopy (Stump et al., 1979). Pellets of approximately 1 cm diameter were prepared by pressing the powder in a 30 Ton Press C-30 machine (Research Industrial Instruments Co., London). As pellet thickness affects the efficiency of the instrument, pellet thicknesses of 50 - 640 mg/cm² were used to determine the optimum efficiency, dead time and acquisition time. Depending on the instrument at the time of measurement, pellets of approximately 160 or 140 mg/cm² thickness were used.

Pellets were loaded in batches of sixteen into a sample chamber, analyzed automatically one at a time for 15 or 30 min using a molybdenum target. Data were collected and viewed on a Nuclear Data (ND-66) analyzer and stored on 9-track magnetic tapes (Memorex MRX V). The peaks of each element were identified by the measured energies and their net areas extracted using software developed in the X-ray laboratory.

Calibration curves for the elemental analyses were prepared from pellets of certified coal, tomato, orchard leaves, bovine liver and oyster tissue of the U.S. National Bureau of Standards Certificate of Analysis (1975-1979). These pellets were run in the same way as the sample pellets and were assumed to have similar absorption characteristics. To ensure their similarity,
all peak areas were normalized against the Compton (inelastic scatter of each spectrum) peak to correct for machine error, different count times etc. Furthermore, each Compton normalized peak area was compared against a calibration curve specific to that element.

After all the samples were run, up to 16 pellets were chosen randomly and tested for variability between the two sides of the pellet and/or reproducibility of the concentration of the elements by comparison with the original runs.

DATA ANALYSIS

As the nematode inoculum level was quantitatively linear, the appropriate analysis was regression (Swallow, 1984). Consequently, the Biomedical Data Processing (BMDP) package (Dixon, 1981) was utilized. However, in cases where interactions were involved, analysis of variance (ANOVA) techniques and the Duncan's and Newman-Keul multiple range tests were used. The F values for the regression analyzed data were calculated as outlined in Zar (1974).

Parts of the Materials and Methods have been published in Melakeberhan et al. (1984) (see Appendix III).
INTRODUCTION

The severity of crop loss due to root-knot nematode is influenced by many biological and physiological factors (Seinhorst, 1961; Wallace, 1969 & 1974; Webster, 1969). For example, nematodes may affect photosynthesis and growth by influencing the host's nutrient uptake and content (Wallace, 1974), translocation of photosynthates (Bird & Loveys, 1975), and transpiration (Evans, 1982). None is more important, however, than the size of the nematode population to which the crop is exposed.

In determining yield loss it is important that the studies use a range of inocula so that more realistic predictions can be made from the data. The type of inoculum used, however, varies with the nematode species and experimenter's preference. Cysts, eggs, egg sacks and/or second stage larvae are commonly used forms of inoculum when estimating the damage done by nematodes to crops (Schmitt & Noel, 1984). However, a difficulty is that
all forms of inocula are influenced by soil type and other environmental factors prior to larval penetration of the host root. Many experimenters prefer to use second stage larvae as the inoculum, as egg hatch is generally very variable.

The host response is influenced by the number of nematodes in the root and the stage of infection (Wallace, 1974; Barker & Oltoff, 1976). It is important to estimate the number and stage of development of nematodes that are established in the host roots. Furthermore, the host response is influenced by the number of generations or part generations of the nematode in the roots and this complicates the evaluation of the mechanisms of loss of yield. Until now, however, nematode induced yield loss estimates have been based on infections involving multiple generations (eg. Kinloch, 1982; Cook, 1984).

The objectives of this study were to determine (1) the relationship between initial inoculum level and the number of nematodes recovered 3 wk after inoculation, (2) the growth, elemental content and physiological responses of *P. vulgaris* to different inoculum levels of *M. incognita* and (3) the relationship between these responses and symptom expression as they may relate to yield.
MATERIALS AND METHODS

Seedlings were transplanted 3 d after germination and were inoculated 4 d later with 0 (control), 1000, 5000 or 10000 freshly hatched *M. incognita* larvae/plant. Each treatment was replicated four times and plants were maintained as described in chapter II.

Plants were harvested 3 wk after inoculation. The number of buds and flowers were counted, and the leaf area was determined by counting the number of millimeter squares occupied by leaves traced onto graph paper. Chlorophyll content was determined on a fresh weight basis. The APS and dark respiration rates were determined over 2 d in a closed system using a URAS II infrared CO₂ analyzer connected to a 1.7 l chamber which enclosed intact shoots of the plants. A chamber air temperature of 22 ± 2°C, and a light irradiance of 750 μE.m⁻².s⁻¹ and air flow rate of 1 l.min⁻¹ were used for the CO₂ exchange measurements.

After samples were taken for estimation of the number of nematodes established in the roots, the remainder of the root and the shoots were dried and processed for C-H-N and other elemental analyses. The concentrations of calcium, copper, iron, manganese, potassium and zinc were determined using X-ray energy spectroscopy from separate pellets of approximately equal thickness (160 mg/cm²) prepared from a pulverized, homogeneous powder. Each pellet, together with similarly prepared pellets of
well-mixed biological standard powders of known elemental concentration was analyzed for 15 min using X-ray energy spectroscopy (Stump et al., 1979).

The experimental data were analysed using regression analysis except for the nutrient elements where ANOVA together with Duncan's multiple range and the Newman-Kuel test were used.

RESULTS

The number of third and fourth stage *M. incognita* larvae recovered from the roots was directly proportional to the initial inoculum level (Fig. 4).

Linear regression analysis showed a significant 

\[ P \leq 0.01 \]

inverse relationship between total, primary and trifoliate leaf area (Fig. 5 a), shoot, root and total plant weight (Fig. 5 b), and the number of flowers and total yield potential (Fig. 5 c) with an increase in nematode infection. The number of closed buds was not significantly affected by the nematode infection.

The total chlorophyll \((a + b)\) and chlorophyll a content (Fig. 6 a) and the APS rate on a leaf area and shoot nitrogen basis (Fig. 6 b) decreased significantly \((P \leq 0.01)\) with increase in level of nematode infection. The chlorophyll b content and APS rate on a total chlorophyll basis did not significantly decrease with increasing nematode infection.
Figure 4
The relationship between initial nematode inoculum level and number of 3rd and 4th stage *Meloidogyne incognita* larvae recovered from *Phaseolus vulgaris* roots 3 wk after inoculation.
$y = 0.074x + 49.15$

$r = 0.97$

Initial inoculum level vs. number of larvae recovered.
Figure 5
The relationship of a) leaf area, b) plant dry weight and c) yield of *Phaseolus vulgaris* plants to different levels of *Meloidogyne incognita* at 3 wk after inoculation.
A

TOTAL LEAF AREA

$y = -0.001x + 1.46$

$r = -0.78$

PRIMARIES

$y = -0.0033x + 0.84$

$r = -0.67$

TRIFOLIATES

$y = -0.001x + 0.62$

$r = -0.81$

B

PLANT TOTAL

$y = -0.002x + 1.82$

$r = -0.88$

SHOOT

$y = -0.001x + 1.28$

$r = -0.88$

ROOTS

$y = -0.0004x + 0.55$

$r = -0.85$

C

BUDS AND FLOWERS

$y = -0.009x + 12.07$

$r = -0.84$

CLOSED BUDS

$y = -0.003x + 6.67$

$r = -0.40$

OPEN FLOWERS

$y = -0.007x + 5.39$

$r = -0.88$

NUMBER OF LARVAE RECOVERED

/g FRESH ROOT WEIGHT
Figure 6

Regressions of a) chlorophyll content, b) photosynthetic rate and c) dark respiration rate of *Meloidogyne incognita* infected *Phaseolus vulgaris* plants at 3 wk after inoculation.
A

CHLOROPHYLL CONTENT (mg) /g LEAF FRESH WEIGHT

TOTAL

CHLOROPHYLL (a+b)

\[ y = -0.002x + 2.59 \]
\[ r = -0.72 \]

CHLOROPHYLL a

\[ y = -0.001x + 2.06 \]
\[ r = -0.78 \]

CHLOROPHYLL b

\[ y = -0.0003x + 0.52 \]
\[ r = -0.30 \]

B

PHOTOSYNTHETIC RATE (mg CO₂·h⁻¹)

TOTAL

CHLOROPHYLL (a+b) /g OF LEAF

\[ y = -0.0003x + 1.78 \]
\[ r = -0.11 \]

mg⁻¹ SHOOT N

\[ y = 0.0003x + 0.25 \]
\[ r = -0.87 \]

C

RESPIRATION RATE (mg CO₂·h⁻¹)

NUMBER OF LARVAE RECOVERED /g FRESH ROOT WEIGHT

\[ y = 0.005x + 1.71 \]
\[ r = 0.85 \]

\[ y = 0.001x + 1.52 \]
\[ r = 0.61 \]
Figure 7

The influence of different levels of *Meloidogyne incognita* infection on the total a) carbon, b) hydrogen, c) nitrogen content and d) carbon to nitrogen ratio of *Phaseolus vulgaris* plants at 3 wk after inoculation.
Respiration rate (on a shoot dry weight and leaf area basis) increased \((P \leq 0.05)\) with nematode infection (Fig. 6 c).

Plant nutrient contents were expressed on a unit weight basis (concentration) relative to values of the standards, and on the total tissue content. Total plant, shoot and root carbon \((P \leq 0.01)\) (Fig. 7 a), total plant, shoot hydrogen \((P \leq 0.01)\), root hydrogen \((P \leq 0.05)\) (Fig. 7 b), total plant nitrogen \((P \leq 0.05)\) and root nitrogen \((P \leq 0.01)\) (Fig. 7 c) content on a per plant basis, and the carbon to nitrogen ratio \((P \leq 0.01)\) (Fig. 7 d) significantly decreased with increasing nematode infection. Shoot nitrogen was not significantly affected by nematodes. There was no relationship between nematode infection and percent carbon, but percent hydrogen of both roots and shoots decreased \((P \leq 0.05)\) while percent nitrogen (roots and shoots) increased \((P \leq 0.05)\) with an increased nematode infection.

The concentrations of calcium, copper and iron in the shoot (Table 1 a) and of potassium in the roots (Table 1 b) increased in plants infected with the largest number of nematodes while those of copper and zinc in the roots were lower \((P \leq 0.05)\) (Table 1 b), compared with the controls. There was no apparent change in the concentration of manganese with nematode infection. When the concentration of the elements was expressed as a shoot:root ratio, only calcium, iron and copper significantly \((P \leq 0.05)\) increased with increasing nematode infection (Table 1 c).
Table 1
The influence of *Meloidogyne incognita* on the concentration of nutrient elements in the (a) shoot and (b) root and on the (c) shoot:root ratio of these elements of *Phaseolus vulgaris* plants at 3 wk after inoculation.

Numbers in each column are relative to the corresponding elemental standards. N.R. = Mean number of nematodes recovered/g fresh root weight. Means followed by the same letter are not significantly different at $P \leq 0.05$ (n=4).

<table>
<thead>
<tr>
<th>Plant Tissue</th>
<th>N.R.</th>
<th>Elements</th>
<th>K</th>
<th>Ca</th>
<th>Mn</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Shoot</td>
<td>0</td>
<td>6.5</td>
<td>8.9 c</td>
<td>0.6</td>
<td>2.3 b</td>
<td>0.3 b</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>5.6</td>
<td>9.7 c</td>
<td>0.6</td>
<td>2.9 b</td>
<td>0.3 b</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>382</td>
<td>7.6</td>
<td>13.2 b</td>
<td>0.8</td>
<td>5.6 a</td>
<td>0.5 a</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>762</td>
<td>8.6</td>
<td>16.0 a</td>
<td>0.9</td>
<td>6.4 a</td>
<td>0.6 a</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>b) Root</td>
<td>0</td>
<td>8.2 b</td>
<td>6.8</td>
<td>1.7</td>
<td>71.8</td>
<td>4.2 b</td>
<td>2.5 a</td>
<td></td>
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<td>156</td>
<td>8.7 b</td>
<td>6.1</td>
<td>1.4</td>
<td>66.0</td>
<td>5.0 a</td>
<td>2.7 a</td>
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<td></td>
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<td>10.0 ab</td>
<td>6.3</td>
<td>1.7</td>
<td>89.2</td>
<td>3.7 bc</td>
<td>2.0 b</td>
<td></td>
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<td>10.8 a</td>
<td>6.2</td>
<td>1.0</td>
<td>66.6</td>
<td>3.2 c</td>
<td>1.6 c</td>
<td></td>
</tr>
<tr>
<td>c) Shoot:root</td>
<td>0</td>
<td>0.79</td>
<td>1.31 c</td>
<td>0.35</td>
<td>0.03 b</td>
<td>0.07 c</td>
<td>0.48</td>
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<td>156</td>
<td>0.64</td>
<td>1.59 c</td>
<td>0.43</td>
<td>0.04 b</td>
<td>0.06 c</td>
<td>0.30</td>
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<tr>
<td></td>
<td>382</td>
<td>0.76</td>
<td>2.10 b</td>
<td>0.47</td>
<td>0.06 ab</td>
<td>0.14 b</td>
<td>0.45</td>
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<tr>
<td></td>
<td>762</td>
<td>0.80</td>
<td>2.58 a</td>
<td>0.90</td>
<td>0.10 a</td>
<td>0.19 a</td>
<td>0.56</td>
<td></td>
</tr>
</tbody>
</table>
The influence of *Meloidogyne incognita* on the total plant elemental content of *Phaseolus vulgaris* plants for (a) shoot, (b) root and (c) total plant, of the (d) shoot:root ratio of these elements and of the (e) shoot elements expressed as a percent of total plant weight at 3 wk after inoculation. Numbers in each column for a), b) and c) are based on total weight. Other details were as in Table 1.

<table>
<thead>
<tr>
<th>Plant Tissue</th>
<th>N.R.</th>
<th>Elements</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K</td>
<td>Ca</td>
</tr>
<tr>
<td>a) Shoot</td>
<td>0</td>
<td>213.3 a</td>
<td>292.2</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>149.0 b</td>
<td>257.6</td>
</tr>
<tr>
<td></td>
<td>382</td>
<td>121.0 b</td>
<td>210.8</td>
</tr>
<tr>
<td></td>
<td>762</td>
<td>104.4 b</td>
<td>189.3</td>
</tr>
<tr>
<td>b) Root</td>
<td>0</td>
<td>120.7 a</td>
<td>100.4 a</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>90.6 ab</td>
<td>64.3 b</td>
</tr>
<tr>
<td></td>
<td>382</td>
<td>87.3 ab</td>
<td>55.1 bc</td>
</tr>
<tr>
<td></td>
<td>762</td>
<td>67.4 ab</td>
<td>38.8 c</td>
</tr>
<tr>
<td>c) Total (Shoot + Root)</td>
<td>0</td>
<td>334.0 a</td>
<td>392.6 a</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>239.6 b</td>
<td>321.9 ab</td>
</tr>
<tr>
<td></td>
<td>382</td>
<td>208.3 b</td>
<td>265.9 b</td>
</tr>
<tr>
<td></td>
<td>762</td>
<td>171.8 b</td>
<td>228.1 b</td>
</tr>
<tr>
<td>d) Shoot: Root</td>
<td>0</td>
<td>1.77</td>
<td>2.91 b</td>
</tr>
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<td></td>
<td>156</td>
<td>1.64</td>
<td>4.01 ab</td>
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<td></td>
<td>382</td>
<td>1.39</td>
<td>3.83 ab</td>
</tr>
<tr>
<td></td>
<td>762</td>
<td>1.55</td>
<td>4.88 a</td>
</tr>
<tr>
<td>e) On Shoot as % of Total</td>
<td>0</td>
<td>63.9</td>
<td>74.4 b</td>
</tr>
<tr>
<td></td>
<td>156</td>
<td>62.2</td>
<td>80.0 ab</td>
</tr>
<tr>
<td></td>
<td>382</td>
<td>58.1</td>
<td>79.3 ab</td>
</tr>
</tbody>
</table>

48
Total shoot content of potassium, manganese and zinc (Table 2a), and root content of all elements was significantly ($P \leq 0.05$) lower in most nematode infected plants, compared with the controls (Table 2b). However, the total (shoot and root) content of the elements measured was significantly ($P \leq 0.05$) lower in the highest nematode infections, compared with the controls (Table 2c). When the total elemental contents were expressed as a shoot:root ratio (Table 2d), and the shoot as a percentage of the total plant weight (Table 2e), the plants infected with the largest number of nematodes contained significantly ($P \leq 0.05$) more calcium and copper than the controls.
DISCUSSION

An understanding of the interaction between nematode and host, as reflected by the host's physiological processes, and the apparent collective influence of the host-parasite systems on crop yield requires concurrent measurement of several morphological and physiological parameters over a range of nematode inocula. Although the number of larvae recovered from the roots increased with initial inoculum level, not all larvae inoculated into the soil invade and mature in the host (Wallace, 1974). It may appear that a curvilinear regression could fit the data (Fig. 4) better than a linear regression. This was not done because an \( r \) value of more than 0.97 but less than 1.0 would have made very little difference in the interpretation.

The values of most physiological and morphological characteristics were significantly less with increasing numbers of *M. incognita* in the root. Exceptions were respiration rate, percent total nitrogen, and the concentrations of calcium, iron and copper in the shoot and of potassium in the root. When the experiment was repeated the results showed similar trends except that the dark respiration rate and percent nitrogen content decreased rather than increased with higher levels of nematode infection. This inconsistency may be due to natural variation between the two batches of plants (Larcher, 1980) or to the
differing behaviour and virulence of the developing larvae.

The general decrease in total chlorophyll content is a reflection of the ageing of the leaves (Sestak, 1977 b). This results in the APS rate decreasing with level of nematode infection, and is a pattern similar to that of other reports (Loveys & Bird, 1973; Wallace, 1974; Bird & Loveys, 1975; Franco, 1980). The decrease in leaf area and shoot weight has significant implications for photosynthesis and crop yield. *M. incognita* develop in the xylem parenchyma of growing roots (Paulson & Webster, 1970). Hence, the lower root weight and presumably their diminished functional capability are likely additional factors contributing to slower growth, decreased flower production and lower crop yield. The parallel significant decrease of carbon and hydrogen is to be expected, in view of the decreased mass of the main structural elements of the plant. In early maturing cultivars, as used in these experiments, ontogenesis normally occurs very rapidly (Sestak, 1977 b). When this is accompanied by the influence of an increased intensity of nematode parasitism, the adverse effect on general growth, particularly the area of the trifoliates and yield potential is likely to be greater.

The percent and total nitrogen content of plants appears to vary with stage of infection, number of developing nematode larvae at any given time and the host type. Hunter (1958), for example, in measuring percent total nitrogen content, showed it decreased in tomatoes whereas Ibrahim *et al.*, (1982) showed it
increased in cotton plants. However, Oteifa (1952), in measuring total nitrogen content, showed it decreased in lima bean whereas Owens & Novotny (1960) showed it increased in tomatoes and cucumber. Baldwin et al., (1979) recorded both an increased and decreased nitrogen fixation in soybeans as a result of nematode infection. In my experiments, the increased percent nitrogen, decreased total nitrogen content and carbon to nitrogen ratio of nematode infected plants suggests that *M. incognita* interferes with nitrogen metabolism in a way that diminishes host productivity. Furthermore, the decline in photosynthetic rate, when expressed on a total shoot nitrogen content basis, suggests that the nitrogen may have been converted to some storage form which plants under stress are known to accumulate (Singh et al., 1978; Fattah & Webster, 1984).

The total plant nutrient content was significantly decreased in *M. incognita* infected plants, presumably due to the effect of the nematode on plant dry weight (Price et al., 1982). However, the effect of *M. incognita* on concentration of these nutrient elements seems to be variable. Thus, calcium, copper and iron in the shoot and potassium in the root increased (Nasr et al., 1980) while copper and zinc in the roots decreased with nematode infection. The distribution of elements within the nematode infected plants showed a larger percentage of calcium, copper and iron in the shoots than in the roots. This implies that translocation of these elements from the site of uptake was not affected by nematode infection.
Potassium and calcium are translocated via the symplastic and apoplastic route respectively (Evans & Franco, 1979) which may explain in our experiments why the concentration of potassium was higher in the root than was calcium. It has been suggested (Evans et al., 1977; Evans & Franco, 1979; Evans, 1982) that the amount of plant calcium may be a good indicator of the tolerance level of plants to nematodes. In these experiments, nematode intolerant plants accumulated more calcium per unit of water transpired which suggests that the amount of calcium in nematode infected plants may be an indicator of direct or indirect nematode damage (Evans & Franco, 1979).

Many of the symptoms and yield reduction in *Meloidogyne* infected French bean plants are the final manifestation of a wide range of interacting physiological processes. It seems likely from the changed concentrations of elements and decreased photosynthesis that nematode infections influence nutrient uptake and transport (Wallace, 1974) (perhaps through modified membrane function) which, in turn, could interfere with chlorophyll production, APS rate and other physiological processes associated with optimum growth.

These results were from samples obtained 3 wk after inoculation, and it is likely that the intensity of the effects of the nematode varies with the duration of the host-parasite interaction. Thus, it was necessary to examine how these physiological processes and morphological characteristics in this short life cycle host were affected over a single
generation of the nematode.

The results of this chapter are published in Melakeberhan et al. (1985 a) (see Appendix III).
CHAPTER IV

THE INFLUENCE OF A SINGLE GENERATION OF *MELIODOGYNE INCOGNITA*

INTRODUCTION

The extent of plant cell damage by root-knot nematodes and their influence on host physiology varies with age of the nematode (Bird, 1974; Bird *et al.*, 1980). Loveys & Bird (1973) reported a significant decrease in photosynthesis due to *M. javanica* infection of tomato plants at intervals over 22 d. A similar study by Wallace (1974), using *M. javanica* infected tomato plants, suggested that a complex of interacting physiological factors affected photosynthesis. In chapter III, using a 3 week-old *M. incognita* infections of bean plants, it was concluded that nematodes influence nutrient uptake and transport which, in turn, likely interfere with chlorophyll production, photosynthesis and other physiological processes associated with plant growth. Since these conclusions are based on a sample from one time period in this developing host-parasite interaction, it was necessary to examine the changes in the host's physiology at different developmental stages of the nematode and within a single generation of its
development.

The experiments reported in this chapter were designed to determine (1) the effect of different inoculum levels of *M. incognita* on the physiology, growth and yield of the bean plants at several stages of maturity during a single generation of the nematode using destructive and non-destructive assays and (2) the relationship between the altered physiology and changing symptom expression of the plant host.

**MATERIALS AND METHODS**

One week after seed germination, individual bean plants were inoculated with 0 (control), 2000, 4000 or 8000 *M. incognita* larvae. The first and second sampling dates (see below) received the required inoculum at the same time while in the rest of the treatments inoculation was spread over 24 hr. Each treatment was replicated four times and plants arranged randomly in the growth chamber.

Morphological and physiological measurements of the bean plants were done using destructive and non-destructive assays. In the destructive assay, four bean plants from each treatment were harvested at 1, 3, 8, 15, 22, and 27 d after nematode inoculation and assessed for APS and dark respiration rates (at $21.5 \pm 1^\circ C$, $750 \mu E.m^{-2}.s^{-1}$ and air flow rate of 4 - 4.5
1 min⁻¹), leaf area, dry weight, bud, flower, pod and seed production, and for total chlorophyll (leaf area basis), carbon, hydrogen and nitrogen content. In the non-destructive assay, one batch of plants was used for the measurement of APS and dark respiration rates, leaf area and for the yield components at 0, 3, 8, 15, 21 and 28 d after inoculation.

The data were analyzed utilizing linear regression as follows: (1) by comparing all treatments at each sampling date as presented in the figures and (2) to determine the change with time the same data were analyzed on a treatment basis (0, 2000, 4000 & 8000 larvae/plant) and the slope and r values are included.

RESULTS

Nematodes invaded the roots within 24 hr of inoculation into the soil and continued to enter the roots for about one week. During the second week, no penetrating second stage larvae were observed but the late second, and third and fourth stage larvae were present in the roots. At this time, abscission of the primary leaves began in the higher inoculum treatments and progressed with time. Three weeks after inoculation the third and fourth larval stages and adults were visible and after 4 wk only adults were present.
Figure 8

The relationship between inoculum level and number of *Meloidogyne incognita* recovered at each sampling date (destructive assay) and at the end of the experiment (non-destructive assay). The data for sampling dates 1 and 3 (top right) are from a repeated experiment for the chlorophyll analysis.
DESTRUCTIVE ASSAY

NON-DESTRUCTIVE ASSAY

INOCULUM LEVEL

NUMBER OF NEMATODES RECOVERED/g FRESH ROOT WEIGHT

1. $y = 0.04x + 13, r = 0.87$
2. $y = 0.02x + 13, r = 0.77$
3. $y = 0.04x + 46, r = 0.78$
4. $y = 0.03x + 57, r = 0.77$
5. $y = 0.13x + 153, r = 0.92$
6. $y = 0.12x + 94, r = 0.96$
7. $y = 0.06x + 136, r = 0.88$
8. $y = 0.03x + 54, r = 0.54$
9. $y = 0.10x + 100, r = 0.51$
Galls on bean plants were visible 3 d after inoculation and increased in size with time.

The number of nematodes recovered at each sampling date was directly proportional to the inoculum level (Fig. 8). However, the numbers recovered at each sampling date did not increase with duration of infection. The maximum numbers recovered were at 2 wk after inoculation.

The values of most growth parameters measured generally decreased with increasing number of nematodes and duration of infection. At 15 d from inoculation, leaf area \((P \leq 0.05)\) (Fig. 9) and after 8 d shoot, root \((P \leq 0.05)\) and total plant weight \((P \leq 0.01)\) (Fig. 10) was significantly less with increasing number of nematodes. Although the overall leaf area and plant weight significantly increased \((P \leq 0.05)\) with time for almost all treatments, the dry weight of nematode treated plants only approximately doubled whereas the weight of the control plants increased almost five times from the time of inoculation (Fig. 10 and Table 3).

The total carbon (Fig. 11), hydrogen (Fig. 12) and nitrogen content (Fig. 13) followed the same decreasing pattern with time of inoculation as did plant weight. The plant total, shoot and root content of all three elements was significantly less \((P \leq 0.05)\) from 8 d after inoculation with increasing number of nematodes. The total content of these elements increased significantly \((P \leq 0.01)\) with time in all treatments but less so
NOTES FOR FIGURES 9-18:

1) All figures are based on the number of *Meloidogyne incognita* larvae and/or adults recovered per g fresh root weight of *Phaseolus vulgaris* after inoculating with 0 (control), 2000, 4000 & 8000 larvae per plant. Unless otherwise specified, the results are from the destructive assays.

2) Circled numbers refer to the days that samples were taken after larval inoculation.

Figure 9

Effect of *Meloidogyne incognita* on total leaf area of *Phaseolus vulgaris* over 27 d in the destructive assay (left) and over 28 d in the non-destructive assay (right).
DESTRUCTIVE ASSAY

NON-DESTRUCTIVE ASSAY

LEAF AREA (dm²)

RECOVERED

INOCULATED

NUMBER OF NEMATODES

0 300 600 900 1200

0 2000 4000 6000 8000

0 300 600 900 1200

62
Figure 10

Effect of *Meloidogyne incognita* on dry weight of bean plants over a 27 d period after nematode inoculation.
NUMBER OF NEMATODES RECOVERED /g FRESH ROOT WEIGHT
with increasing number of nematodes (Fig. 11 - 13 and Table 3). Carbon and hydrogen (percent per unit weight basis) showed a decreasing trend with time, but was significantly ($P \leq 0.05$) so only towards the end of the experiment whereas shoot nitrogen increased ($P \leq 0.05$) with number of nematodes from 15 d after infection (Table 4 & 5). The total C:N and H:N ratios tended to decrease with increasing number of nematodes and were significantly ($P \leq 0.05$) greater after 15 d (Table 4).

Leaf samples collected for chlorophyll analysis on dates 1 and 3 (destructive assay) were lost due to an electrical failure. Therefore, data given for 1 and 3 d are from a separate repeated experiment. Total chlorophyll (a and b) content (Fig. 14) for all treatments reached an apparent maximum at 3 d after inoculation, coinciding with the time of maximum primary leaf expansion, and then generally decreased but more so with increasing level of nematode infection (Table 6). However, there was a significant decrease with increasing number of nematodes at only 15 d ($P \leq 0.05$) and 27 d ($P \leq 0.01$) after the initial infection.

The APS rate based, on the total chlorophyll content at 15 d and shoot nitrogen content at 15 and 22 d, decreased significantly ($P \leq 0.05$) with increasing number of nematodes (Fig. 15). On an inoculum level basis, however, it decreased for 8 d after inoculation. From 8 d, the APS rate on a leaf area basis (Fig. 16) decreased significantly ($P \leq 0.05$) with inoculum
Figure 11

Effect of *Meloidogyne incognita* on total carbon content of *Phaseolus vulgaris* over a 27 d period after nematode inoculation.
A

TOTAL

- SHOOT

- ROOT

NUMBER OF NEMATODES RECOVERED /g FRESH ROOT WEIGHT

TOTAL CARBON CONTENT (mg)
Figure 12
Effect of *Meloidogyne incognita* on total hydrogen content of *Phaseolus vulgaris* over a 27 d period after nematode inoculation. Please note the two different Y scales.
Figure 13
Effect of *Meloidogyne incognita* on total nitrogen content of *Phaseolus vulgaris* plants over a 27 d period after nematode inoculation.
Regression equations of the changes in leaf area (dm²), total plant weight (g) and total carbon, hydrogen and nitrogen content (mg) of bean plants infected with four levels of *Meloidogyne incognita* over 28 d after inoculation.

Unless specified otherwise, results are from the destructive assay experiment.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>LEAF AREA</th>
<th>TOTAL PLANT WEIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-destructive</td>
<td>Destructive assay</td>
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<tr>
<td></td>
<td><em>y</em></td>
<td><em>r</em></td>
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<tr>
<td>0</td>
<td>0.06X + 0.80 0.78</td>
<td>0.03X + 0.76 0.61</td>
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<tr>
<td>2000</td>
<td>0.03X + 0.87 0.55</td>
<td>0.02X + 0.75 0.52</td>
</tr>
<tr>
<td>4000</td>
<td>0.01X + 0.83 0.37</td>
<td>0.01X + 0.75 0.45</td>
</tr>
<tr>
<td>8000</td>
<td>0.01X + 0.91 0.21</td>
<td>0.01X + 0.79 0.32</td>
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<table>
<thead>
<tr>
<th>CARBON</th>
<th>HYDROGEN</th>
<th>NITROGEN</th>
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<tr>
<td><em>y</em></td>
<td><em>r</em></td>
<td><em>y</em></td>
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<tr>
<td>0 31.5X + 153.2 0.87</td>
<td>4.5X + 22.5 0.87</td>
<td>8.47X + 6.7 0.86</td>
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<tr>
<td>2000 10.3X + 216.3 0.72</td>
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<td>3.20X + 14.9 0.63</td>
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<tr>
<td>4000 7.1X + 210.4 0.60</td>
<td>0.9X + 31.0 0.52</td>
<td>2.05X + 16.1 0.52</td>
</tr>
<tr>
<td>8000 5.6X + 214.5 0.56</td>
<td>0.7X + 29.3 0.53</td>
<td>1.70X + 16.9 0.52</td>
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</table>
Table 4

Regression equations of the total percent shoot and root carbon, hydrogen and nitrogen and the total carbon to nitrogen and hydrogen to nitrogen ratios of bean plants over a 27 d after inoculation with *Meloidogyne incognita*.

<table>
<thead>
<tr>
<th>DAYS AFTER INOCULATION</th>
<th>SHOOT</th>
<th></th>
<th>ROOT</th>
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<tbody>
<tr>
<td></td>
<td><em>y</em></td>
<td><em>r</em></td>
<td><em>y</em></td>
<td><em>r</em></td>
</tr>
<tr>
<td>1</td>
<td>-0.0004X + 40.9</td>
<td>-0.10</td>
<td>0.0043X + 22.8</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>-0.0001X + 40.9</td>
<td>-0.02</td>
<td>-0.0064X + 30.0</td>
<td>-0.16</td>
</tr>
<tr>
<td>8</td>
<td>-0.0013X + 41.0</td>
<td>-0.41</td>
<td>0.0070X + 30.5</td>
<td>0.27</td>
</tr>
<tr>
<td>15</td>
<td>-0.0010X + 40.4</td>
<td>-0.65</td>
<td>0.0011X + 31.9</td>
<td>0.23</td>
</tr>
<tr>
<td>22</td>
<td>-0.0003X + 39.6</td>
<td>-0.04</td>
<td>0.0012X + 34.0</td>
<td>0.25</td>
</tr>
<tr>
<td>27</td>
<td>-0.0008X + 40.2</td>
<td>-0.57</td>
<td>-0.0003X + 35.0</td>
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<tr>
<td></td>
<td><em>y</em></td>
<td><em>r</em></td>
<td><em>y</em></td>
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<td>8</td>
<td>0.0002X + 5.8</td>
<td>0.12</td>
<td>0.0016X + 4.1</td>
<td>0.42</td>
</tr>
<tr>
<td>15</td>
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<td>-0.40</td>
<td>0.0002X + 4.4</td>
<td>0.26</td>
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<tr>
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<td>0.0002X + 3.3</td>
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<td>0.66</td>
<td>-0.0002X + 3.4</td>
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<td>0.0003X + 3.3</td>
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<td>0.0001X + 3.2</td>
<td>0.09</td>
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C:N H:N

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<th><em>y</em></th>
<th><em>r</em></th>
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<td>0.14</td>
<td>-0.0007X + 1.9</td>
<td>0.77</td>
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</tbody>
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73
Table 5

Regression equations of the percent total shoot and root carbon, hydrogen and nitrogen and the carbon to nitrogen and hydrogen to nitrogen ratios of bean plants over a 27 d period after inoculation with *Meloidogyne incognita*

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>CARBON</th>
<th>HYDROGEN</th>
<th>NITROGEN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( y = -0.01x + 4)</td>
<td>( y = 0.0001x + 5.9)</td>
<td>( y = 0.063x + 4.6)</td>
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<tr>
<td>2000</td>
<td>( y = -0.02x + 4)</td>
<td>( y = -0.0049x + 5.9)</td>
<td>( y = -0.020x + 4.2)</td>
</tr>
<tr>
<td>4000</td>
<td>( y = -0.10x + 4)</td>
<td>( y = -0.0156x + 6.0)</td>
<td>( y = -0.019x + 4.4)</td>
</tr>
<tr>
<td>8000</td>
<td>( y = 0.35x + 5)</td>
<td>( y = 0.0005x + 5.7)</td>
<td>( y = 0.007x + 4.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>ROOT</th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( y = 0.34x + 2)</td>
<td>( y = 0.0400x + 3.8)</td>
<td>( y = 0.002x + 3.3)</td>
</tr>
<tr>
<td>2000</td>
<td>( y = 0.35x + 2)</td>
<td>( y = 0.0400x + 3.8)</td>
<td>( y = 0.020x + 2.9)</td>
</tr>
<tr>
<td>4000</td>
<td>( y = 0.32x + 2)</td>
<td>( y = 0.0400x + 3.7)</td>
<td>( y = 0.016x + 3.0)</td>
</tr>
<tr>
<td>8000</td>
<td>( y = 0.34x + 2)</td>
<td>( y = 0.0400x + 3.9)</td>
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C:N

<table>
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<th>( y = 0.17x + 8.4)</th>
<th>( y = 0.0200x + 1.3)</th>
<th>( y = 0.80)</th>
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<tbody>
<tr>
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<td>( y = 0.34)</td>
</tr>
<tr>
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<td>( y = -0.0003x + 1.4)</td>
<td>( y = -0.001)</td>
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<tr>
<td>8000</td>
<td>( y = 0.05x + 8.9)</td>
<td>( y = 0.0030x + 1.3)</td>
<td>( y = 0.18)</td>
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</tbody>
</table>

H:N

74
level and/or on number of nematode basis for both assays except for the last sampling date in the non-destructive assay. The APS rate (on a leaf area, total chlorophyll and shoot nitrogen content basis) of all treatments generally decreased significantly ($P \leq 0.01$) with duration of infection (Fig. 15 & 16 and Table 6).

Respiration rate (leaf area basis), did not have a consistent trend with increasing number of nematodes (Fig. 17 and Table 6). On a dry weight basis, the respiration rate tended to increase with number of nematodes but significantly so ($P \leq 0.05$) only on the last sampling date.

Buds, flowers, pods and seeds first appeared on the first, third and fourth week after inoculation, respectively. The total number of flowers or buds were significantly ($P \leq 0.01$) fewer from 15 d after inoculation (Fig. 18) and, at the final harvest, pods and seeds ($P \leq 0.01$) and seeds per pod ($P \leq 0.05$) were fewer with increasing inoculum level and/or number of nematodes. To avoid the risk of second generation nematode infection, both destructive and non-destructive assays were terminated at the end of the fourth week when seeds were just being formed. Consequently, seed dry weights were not obtained.
Figure 14

Effect of *Meloidogyne incognita* on chlorophyll content of *Phaseolus vulgaris* plants over a 27 d period after nematode inoculation.
Figure 15

Effect of *Meloidogyne incognita* on the photosynthetic rate on a total chlorophyll (left side) and on a total shoot nitrogen content basis (right side) and the number of nematodes recovered over a 27 d period after inoculation.
Figure 16

Effect of *Meloidogyne incognita* on the APS rate (leaf area basis) of *Phaseolus vulgaris* over a 27 d (destructive assay), left side, and 28 d (non-destructive assay) period, right side, after nematode inoculation.
Figure 17
Effect of Meloidogyne incognita on the dark respiration rate (dry weight and leaf area basis) of Phaseolus vulgaris over 27 d (destructive assay), two left columns, and on a leaf area basis over a 28 d period (non-destructive assay), right column, after nematode inoculation.
Table 6

Regression equations showing the change in total chlorophyll content (mg/dm²), photosynthetic rate on chlorophyll, shoot nitrogen and leaf area basis and dark respiration (leaf area basis) over a duration of 27 d after inoculation of bean plants with four levels of *Meloidogyne incognita*.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>TOTAL CHLOROPHYLL APS RATE</th>
<th>APS RATE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorophyll basis</td>
<td>Shoot nitrogen basis</td>
</tr>
<tr>
<td></td>
<td>y</td>
<td>r</td>
</tr>
<tr>
<td>0</td>
<td>-0.02X + 5.0</td>
<td>-0.08X + 3.0</td>
</tr>
<tr>
<td>2000</td>
<td>-0.42X + 5.6</td>
<td>-0.07X + 2.5</td>
</tr>
<tr>
<td>4000</td>
<td>-0.10X + 5.5</td>
<td>-0.54X + 2.4</td>
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<tr>
<td>8000</td>
<td>-0.08X + 4.7</td>
<td>-0.50X + 2.1</td>
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**DESTRUCTIVE ASSAY**

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</thead>
<tbody>
<tr>
<td>y</td>
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<td>0</td>
</tr>
<tr>
<td>2000</td>
</tr>
<tr>
<td>4000</td>
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<tr>
<td>8000</td>
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</table>

**NON-DESTRUCTIVE ASSAY**

<table>
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<th>APS RATE (Leaf area basis)</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>2000</td>
</tr>
<tr>
<td>4000</td>
</tr>
<tr>
<td>8000</td>
</tr>
</tbody>
</table>

**DARK RESPIRATION RATE (Leaf area basis)**

| y | r |
| 0 | 0.010X + 1.3 | 0.26 |
| 2000 | 0.002X + 1.3 | -0.04 |
| 4000 | 0.006X + 1.3 | 0.11 |
| 8000 | 0.006X + 1.5 | -0.15 |

| y | r |
| 0 | 0.0005X + 1.1 | 0.01 |
| 2000 | 0.0018X + 1.2 | 0.03 |
| 4000 | -0.0130X + 1.3 | -0.38 |
| 8000 | -0.0050X + 1.3 | -0.14 |
Figure 18
Effect of *Meloidogyne incognita* on four different yield components of *Phaseolus vulgaris* over a 27 d (destructive assay), left side, and 28 d period (non-destructive assay), right side, after nematode inoculation.
DISCUSSION

The results of previous APS rate studies of nematode infected plants have been limited by the absence of associated yield data (Loveys & Bird, 1973; Wallace, 1974) or because the research was a multiple generation study (Franco, 1980). The present study shows the effect of a single generation of M. incognita on photosynthesis and seed production. By limiting the study to one generation, interacting secondary biotic factors that might modify the effect of the primary pathogen, and thereby complicate quantification and interpretation can be minimized. A better understanding of the influence of the nematode on its host plant yield can be obtained by measuring several physiological and morphological parameters concurrently using a range of nematode inocula, and comparing the effects of a single with several generations of the parasite.

The number and the speed at which nematodes invade roots is dependent on the host and nematode species, the conditions at the soil-root interface and on energy reserves in the nematode. No larvae were observed penetrating the roots after 8 d following inoculation. However, under less favourable conditions larval invasion may take longer (De Guiran & Ritter, 1979). The number of nematodes I recovered at each sampling date increased with the level of nematode inoculum. The number of nematodes
recovered from the roots increased with time, which is to be expected up to the end of invasion period. However, sampling error is to be expected (Ferris, 1984) and this may account for the variability that exists in the first three sampling days and in the appearance of the slopes over time (Fig. 8). Nonetheless, all measured parameters showed a similar trend and level of significance when expressed on the basis of inoculum level or number of nematodes. The only difference was that of photosynthesis on a total chlorophyll and nitrogen content basis where significant effects occurred 8 d after inoculation (inoculum level basis) as opposed to 15 d when expressed on the basis of number of nematodes.

With the exception of respiration rate and percent nitrogen content, there was generally a significant negative relationship between all measured parameters and increasing number of nematodes. Leaf area was significantly less after 15 d and plant dry weight, primarily due to shoot weight, from 8 d after inoculation with increasing number of nematodes. The significant parallel trend in total carbon and hydrogen, components of the main structural elements of the plant, is to be expected in view of the smaller biomass of the infected plants.

If a plant is to grow and reproduce according to its genetic potential, it has to photosynthesize normally. However, in the *M. incognita* infected plants the APS rate decreased from 8 d after inoculation. The fact that photosynthesis on a total chlorophyll basis decreased before the actual chlorophyll
content decreased suggests that some physiological processes might be progressively interfering with some of the photosystem functions and consequently, diminishing photosynthesis. Similarly, the decrease in C:N and H:N ratios of nematode infected plants suggest that *M. incognita* might be interfering with nitrogen metabolism. Furthermore, the increase in the concentration of nitrogen (percent per unit of plant weight basis) at later stages of infection but a decrease in the overall plant nitrogen content suggests that some of the nitrogen may occur in a storage form (Fattah & Webster, 1984) because the infected plants are under stress (Meon *et al.*, 1978; Singh *et al.*, 1978). As early as one week after inoculation, APS rate (on a total chlorophyll and shoot nitrogen content basis) declined and was followed by leaf chlorosis and abscission. Such physiological changes could be expected to have a significant effect on organization of the photosynthetic apparatus (Jenkins, *et al.*, 1981 a & b; Markides & Goldwaite, 1981) and/or the photosynthetic process (Woodward, 1976; Catsky, *et al.*, 1981; Kutik, *et al.*, 1984). This contributes to the chlorosis symptoms and impedes host development. From these observations, the ageing process in French bean appears somewhat parallel to that of tomato as shown by Mjuge and Estey (1978) in that physiological ageing is induced in the whole plant from the nematode infection site.

No major change in host plant respiration rate occurred from the time of infection, a point noted also by Bird and
Millerd (1962). The effect of *M. incognita* on host respiration rate appears to be influenced by the behaviour, virulence and stage of development of nematode larvae, and is not as consistent as the effect on photosynthesis. It is assumed in these experiments that larval respiration in the roots is not influencing host respiration as monitored through the shoot.

Host age may greatly influence the host-parasite interaction (Webster, 1969). The peak chlorophyll content and APS rate for non-infected *P. vulgaris* plants occurs at the fully expanded primary leaf stage and then characteristically declines except for slight increases at pod and seed maturation (see also Fraser & Bidwell, 1974; Strnadova & Sestak, 1974; Catsky *et al.*, 1976; Ticha *et al.*, 1980; Jenkins & Woolhouse, 1981). When the primary leaves were fully expanded under the given experimental conditions, however, three important events occurred either in sequence or in parallel: (1) the appearance of trifoliate leaves, (2) followed 2-3 d later by the buds and flowers and (3) nematode development reached the late, swollen, second larval stage. We know that giant cell metabolic activity increases with age of the nematode up to the end of egg laying (Paulson & Webster, 1972; Bird, 1979; Fattah, 1983). However, primary leaves of the French beans were already affected before the trifoliate leaves developed and were not able to support the actively growing tissues and buds. The result is a lower yield as expressed by a smaller plant biomass, and fewer flowers and a delayed flowering and seed production. Furthermore, the
physiological data support earlier contentions that when a host is adapted to advantageous though short-lived situations (Sestak, 1977 a & b; Larcher, 1980), and the plant is affected early in its development (as was the case here) and the effect intensifies with time, the capacity and rate of development of the plant slows (Webster, 1975; Dropkin, 1980). Thus, the host plant is less able to sustain a prolonged infestation and at the same time yield according to its genetic potential. This also explains why it was possible with this host cultivar to observe symptoms in the early stage of infection.

At this point it is not clear what enzyme systems or precisely what other physiological processes M. incognita affects in its gross modification of host physiology and seed production. However, the effect of the root-knot nematode on the growth and physiological processes measured here generally increased as the nematode ages. This raises the necessity, therefore, for studying the response of plants of different age at the time of infection to a single generation of the nematode and, thereby, obtain a more complete picture as to how the nematode influences the host's physiology.

The data in this chapter, on inoculum level basis, are in press (see Melakeberhan et al., 1985 b and Appendix III).
CHAPTER V

EFFECT OF A SINGLE GENERATION OF *MELOIDOGYNE INCognita* ON THE
RESPONSE OF PLANTS OF DIFFERENT AGE

INTRODUCTION

Decreases in crop yield depend on the host-parasite interaction, stage of infection and their combined influence on the morphological characteristics and physiological processes of yield formation (Loomis & Adams, 1980). In chapters III and IV, values of most physiological processes and morphological characteristics of the plants showed an inverse relationship with increasing number of nematodes due to infection by a single generation of *M. incognita*. These effects of nematode attack showed increasing severity with level and duration of infection from one week after inoculation.

In determining a host response to infection, however, it is important to consider normal plant ontogeny and, in particular, the three characteristic growth phases (Salisbury & Ross, 1978): (1) the initial logarithmic phase, where plant growth increases exponentially, (2) the linear phase, where growth is relatively constant with time and finally (3) the senescence phase, where
the rate of plant growth decreases as senescence progresses. Under field conditions, nematode infection may occur at any of these phases of host development and do so with differing intensity and frequency depending on the factors such as soil microclimatic conditions and population size of the nematode. Decrease in crop yield is affected accordingly. However, this has not been examined physiologically and its effects quantified. In this chapter the objectives were to investigate the influence of different intensities of a single generation of *M. incognita* on the physiological processes and morphological characteristics of French bean plants which had been inoculated at different stages of maturity.

MATERIALS AND METHODS

In separate experiments, 3-, 11- and 13-day-old *P. vulgaris* plants were inoculated before full primary leaf expansion (BPLE), at the first appearance of trifoliate leaves (TRIF) and at the flower bud (BDS) stages respectively, with 0 (control), 2000, 4000 or 8000 *M. incognita* larvae. Each experiment had four or more replications and plants were arranged randomly in the growth chamber.

In each experiment, the APS and dark respiration rates (at 22 ± 1°C, 750 μE.m⁻².s⁻¹ and air flow rate of 4.5 l.min⁻¹) and
leaf area were measured for all replicates at each sampling date and the number of buds, flowers and/or pods on each plant were counted where present at each of 0 (just before inoculation), 3, 5, 9, 13 and 20 d in the BPLE plants, and at 0, 3, 7, 14 and 21 d in the TRIF and BDS plants. The plant weight, number of seeds/plant and chlorophyll content were measured at 27 d (BPLE) and 28 d (TRIF and BDS) after nematode inoculation.

The data were analyzed as outlined in chapter IV.

RESULTS

The nematode infected roots were severely galled and the infection was concentrated mainly on 30-40 % of the root system nearest to the stem (Fig. 19). This made it difficult to obtain reliable samples to estimate the number of nematodes in the roots.

Although the effect of the nematodes on leaf area tended to increase with level and duration of infection in all plants, leaf area was significantly ($P \leq 0.05$) less with increasing number of nematodes in only the BPLE plants from the second week onward (Fig. 20). Total leaf area, however, generally increased over the duration of the experiment until primary leaf abscission (2 - 3 wk after inoculation) but the degree of increase was less in the higher nematode treatments (Fig 20 and
Table 7).

Chlorophyll content, measured at the end of each experiment, was markedly lower in plants inoculated at the bud stage than for either the BPLE or TRIF plants (Fig. 21). While chlorophyll b content did not change significantly in any of the inoculation stages, total chlorophyll (a + b) in the TRIF and BDS plants ($P \leq 0.01$) and chlorophyll a content in all experiments significantly decreased with increasing inoculum size (Fig. 21).

The APS rate decreased significantly ($P \leq 0.01$) as early as 3 d after inoculation for all nematode treatments on the BPLE plants and continued to significantly decrease with number of nematodes for up to 20 d after inoculation (Fig. 22). For all sampling dates, the APS rate decreased with increasing level of infection. On the TRIF and BDS plants the APS rate was significantly decreased ($P \leq 0.05$) with level of nematode inoculum 7 d after inoculation. This decreased rate generally increased with level and duration of infection more on the TRIF than on BDS plants for up to 21 d after nematode inoculation (Fig. 22). The APS rate decreased significantly ($P \leq 0.01$) with age in all plants for up to 3 wk after inoculation and was generally lowest in plants with the highest infection levels (Fig. 22 and Table 7).

There was no significant relationship between level of nematode inoculum and dark respiration rate (on a leaf area basis) at any of the sampling dates. However, the ratio of APS
to respiration rate significantly decreased \((P \leq 0.05)\) with increasing inoculum level in all experiments as early as 3 d after inoculation, and was more pronounced in the BPLE and TRIF than in the BDS plants at most sampling dates (Fig. 23). This ratio decreased significantly \((P \leq 0.01)\) with time for most sampling dates but the amount of decrease was greater with level of inoculum and age of plants at inoculation (Fig. 23 and Table 7).

At the final harvest, total plant weight was significantly less with increasing inoculum level in the BPLE and TRIF \((P \leq 0.01)\), and BDS plants \((P \leq 0.05)\) (Fig. 24). This response was due more to the significantly lower shoot dry weight than to root dry weight which did not change significantly with level of nematode infection.

The yield, as measured by the number of buds and flowers, was significantly lower with increasing levels of nematode infection from the second week after inoculation in the BPLE \((P \leq 0.05 - 0.01)\), from 21 d in TRIF \((P \leq 0.001)\) and at 14 and 28 d after inoculation in the BDS plants \((P \leq 0.05)\) (Fig. 25). At 27 d after inoculation, the number of buds on the BPLE plants was not affected while the number of flowers was significantly less \((P \leq 0.001)\) as the number of nematodes increased. The number of pods, seeds and seeds/pod in the TRIF plants were significantly fewer \((P \leq 0.001)\) with increasing levels of nematode inoculum, but only pods and seeds were significantly less \((P \leq 0.05)\) in the BDS plants.
Severity of root gall formation and the degree of non-uniformity of *Phaseolus vulgaris* root infection at 28 d after inoculation with *Meloidogyne incognita* larvae (on the left, 8000 larvae; on the right, 2000 larvae).

Note that the top part of the photograph is a magnification of the proximal part of the root system, the region of intensive galling.
NOTES ON FIGURES 20 - 25

(1) All figures show the effect of four inoculum levels of *Meloidogyne incognita* larvae on *Phaseolus vulgaris* plants of different ages.

(2) Plant drawings in each figure show diagramatically the age of the plant at time of nematode inoculation, namely before primary leaf expansion (BPLE), at the trifoliate (TRIF) and bud appearance (BDS) stages.

(3) Circled numbers indicate the number of sampling days after larval inoculation; numbers between graphed data refer to both sets of data.

Figure 20

Effect of *Meloidogyne incognita* on total leaf area of *Phaseolus vulgaris* plants of different age over 27 d (destructive assay), on the left, and 28 d (non-destructive assay), the two right columns, after nematode inoculation.
NEMATODE INOCULUM

LEAF AREA (cm²)

0

4000

8000

y = \frac{8.6 \times 10^{-6}X + 0.10}{r = -0.14}

100
Figure 21

Effect of *Meloidogyne incognita* on chlorophyll content of *Phaseolus vulgaris* plants of different ages 4 wk after nematode inoculation.
Figure 22

Influence of *Meloidogyne incognita* on photosynthetic rate of different age bean plants over a 27, on the left, and 28 d period, the two right columns, after nematode inoculation.
Figure 23
Effect of *Meloidogyne incognita* on the ratio of photosynthetic to respiration rate of *Phaseolus vulgaris* plants of different ages over 27, left column and 28 d period, two right columns, after nematode inoculation.
NEMATODE INOCULUM

PHOTOSYNTHETIC RATE / RESPIRATION RATE RATIO

0

\[ y = -2.9 \times 10^{-6}X + 0.54 \]
\[ r = 0.03 \]

3

\[ y = -6.5 \times 10^{-4}X + 1.56 \]
\[ r = 0.87 \]

5

\[ y = -6.5 \times 10^{-4}X + 1.47 \]
\[ r = 0.72 \]

9

\[ y = -6.7 \times 10^{-4}X + 9.5 \]
\[ r = 0.83 \]

13

\[ y = -6.3 \times 10^{-4}X + 5.9 \]
\[ r = 0.66 \]

20

\[ y = -2.4 \times 10^{-4}X + 2.8 \]
\[ r = 0.39 \]

27

\[ y = -1.5 \times 10^{-4}X + 2.8 \]
\[ r = 0.39 \]

0

\[ y = -4.3 \times 10^{-4}X + 15.4 \]
\[ r = 0.39 \]

3

\[ y = -2.5 \times 10^{-4}X + 7.9 \]
\[ r = 0.56 \]

5

\[ y = -4.8 \times 10^{-4}X + 13.4 \]
\[ r = 0.59 \]

9

\[ y = -5.2 \times 10^{-4}X + 11.4 \]
\[ r = 0.46 \]

13

\[ y = -5.7 \times 10^{-4}X + 9.4 \]
\[ r = 0.53 \]

20

\[ y = -2.5 \times 10^{-4}X + 2.5 \]
\[ r = 0.46 \]

27

\[ y = -3.5 \times 10^{-4}X + 6.7 \]
\[ r = 0.47 \]

0

\[ y = 2.4 \times 10^{-3}X + 11.5 \]
\[ r = 0.03 \]

3

\[ y = -4.3 \times 10^{-4}X + 15.4 \]
\[ r = 0.39 \]

5

\[ y = -4.8 \times 10^{-4}X + 13.4 \]
\[ r = 0.59 \]

9

\[ y = -5.2 \times 10^{-4}X + 11.4 \]
\[ r = 0.46 \]

13

\[ y = -5.7 \times 10^{-4}X + 9.4 \]
\[ r = 0.53 \]

20

\[ y = -2.5 \times 10^{-4}X + 2.5 \]
\[ r = 0.46 \]

27

\[ y = -3.5 \times 10^{-4}X + 6.7 \]
\[ r = 0.47 \]
Regression equations of the change in leaf area (dm²), photosynthetic (APS) rate (mg CO₂.dm⁻².hr⁻¹) and the ratio of photosynthetic to respiration rate of bean plants of different age over a 28 d period after inoculation with four levels of *Meloidogyne incognita*. (BPLE = before primary leaf expansion, TRIF = trifoliate stage and BDS = at the flower bud stage)

<table>
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<tr>
<th>LEAF AREA</th>
<th>TREATMENT</th>
<th>BPLE</th>
<th>TRIF</th>
<th>BDS</th>
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<tr>
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<td>y</td>
<td>r</td>
<td>y</td>
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<td>0.41</td>
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<td>-0.36</td>
</tr>
<tr>
<td>8000</td>
<td>0.007X + 0.57</td>
<td>0.20</td>
<td>-0.011X + 1.29</td>
<td>-0.26</td>
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</tbody>
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<table>
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<th>APS RATE</th>
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<th>BDS</th>
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<td>y</td>
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<td>y</td>
<td>r</td>
<td>y</td>
</tr>
<tr>
<td>0</td>
<td>-0.21X + 11.8</td>
<td>-0.42</td>
<td>-0.20X + 10.2</td>
<td>-0.55</td>
</tr>
<tr>
<td>2000</td>
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<td>-0.50</td>
<td>-0.21X + 8.8</td>
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<td>-0.30X + 9.4</td>
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<td>-0.21X + 8.4</td>
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<td>-0.32X + 9.0</td>
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<table>
<thead>
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<th>TRIF</th>
<th>BDS</th>
</tr>
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<tr>
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<td>y</td>
<td>r</td>
<td>y</td>
</tr>
<tr>
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<td>-0.27X + 11.8</td>
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<td>-0.35X + 13.0</td>
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<td>-0.28X + 10.4</td>
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<tr>
<td>4000</td>
<td>-0.26X + 8.8</td>
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<td>-0.34X + 10.0</td>
<td>-0.75</td>
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<tr>
<td>8000</td>
<td>-0.18X + 7.0</td>
<td>-0.42</td>
<td>-0.31X + 9.1</td>
<td>-0.72</td>
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</tbody>
</table>
Figure 24
Influence of *Meloidogyne incognita* on plant dry weight of *Phaseolus vulgaris* plants of different ages at the end of each experiment.
Plant Total —— Shoot —— Root

---0

NEMATODE INOCULUM

---0
Figure 25

Effect of *Meloidogyne incognita* on yield components of *Phaseolus vulgaris* plants of different ages over a 27 d, left column, and 28 d period, two right columns, after nematode inoculation.
LEGEND

- Total Buds and Flowers
- Buds
- Flowers
- Pods
- Seeds
- Seeds/Pod

NUMBER OF BUDS, FLOWERS, SEEDS, SEEDS OR SEEDS/POD

NEMATODE INOCULUM
DISCUSSION

In assessing the effect of a pathogen on crop yield, it is important that a range of physiological parameters are compared at different times and levels of disease intensity in the growth cycle thereby simulating a range of intensity and timing of interacting pathogens (Gaunt, 1980). Most nematode-induced loss assessments (e.g. Paruthi & Bhatti, 1981; Kinloch, 1982; Cooke, 1984) do not include study of the physiological basis for loss of yield. It has been known for many years that younger plants are affected more than older plants by nematode attack (Seinhorst, 1961), but the responses have not been related to the host's physiology to show how flower or seed production is influenced by a particular duration and intensity of nematode infection.

In these experiments, the distribution pattern of the root galling was such that no reliable estimate could be made of the number of nematodes in the roots. Hence, the influence of nematodes on the plants was based on the size of the initial inoculum levels. However, previous experiments (Figures 4 & 8) showed that there was a linear relationship between inoculum level and the number of nematodes recovered over the range of inocula used in these experiments. It is reasonable, therefore, to assume a similar relationship in the experiments described in
this section. The precise timing of nematode penetration varies with many factors and occurs over several days following soil inoculation. Therefore, the approximate time of inoculation in relation to age of the plant is the determining factor.

In chapters III and IV where bean plants were inoculated one week after seed germination, the APS rate on a leaf area, chlorophyll and total nitrogen content basis declined one week after nematode inoculation, but leaf area generally was not affected until the third week. The overall effect of the nematodes infection on most of the morphological characteristics and physiological processes increased in most nematode treatments with duration and level of infection. The present results provide an additional perspective because of the differing ages of the host plant at the time of initial infection. Leaf area of the infected plants was significantly less when inoculation was at the BPLE but not when it occurred at later stages. The root weight did not decrease relative to that of the control as the level of nematode inoculum increased probably due to increased root-galling. However, accompanying this galling is likely a grossly altered root physiology that influences the translocation and photosynthetic processes which, in turn, lead to a smaller plant biomass production.

The ontogeny of the French bean host and parasitising nematode has been discussed in detail in chapter IV. The peak APS rate and chlorophyll content in French bean occurs at about the time of full expansion of the primary leaves and declines
thereafter. This peak occurred immediately following nematode inoculation for the BPLE and before inoculation for the TRIF and BDS plants in the experiments reported here. It appears, therefore, that the decline in overall chlorophyll content with host age reflects the growth pattern of the plant. As shown in Fig. 22 the APS rate of the BPLE plants declined 3 d after infection and increased with level of infection whereas in the TRIF plants this decrease was not observed until one week after inoculation and was not of the same magnitude. The APS rate of the BDS plants suffered less from nematode infection than did plants inoculated at younger stages. This supports earlier field observations and shows the mechanism in the plant that leads to the expectation that total yield to decrease less when nematodes attack older plants (eg. at the TRIF and BDS plants). The variable photosynthetic rates of the different plants at different sampling dates may reflect variations in the plant's response to parasitism by the nematode as a result of interaction between stimulatory and inhibitory processes in the plant (Wallace, 1971 & 1974).

The balance between the photosynthetic and respiration processes is crucial for normal plant growth. The present results show that respiration rate did not change significantly on a leaf area basis throughout the experimental period. However, in view of the lower biomass of the infected plants the ratio of APS to respiration rate was used to assess the CO₂ balance of the plant. The significant decrease of the ratio with
duration and level of infection appears to be due to the effect of *M. incognita* on photosynthesis rather than on respiration.

The effect of nematodes on the physiological processes and morphology are seen in the final crop yield. The decrease in overall production of total buds and flowers with increasing inoculum level and duration of infection parallels the APS rate trend. The decrease in productivity measured at the end of each experiment, however, varied with age of the host at the time of inoculation. There was no significant relationship between nematode inoculum and the number of closed buds in the BPLE plants but the number of flowers were significantly decreased with increasing inoculum level. Thus, *M. incognita* not only decreased the overall flower production relative to the control but also delayed flowering, a phenomenon which has been observed recently in *Meloidogyne* infected flue-cured tobacco (Rich and Barker, 1984). As shown by the number of pods, seeds and/or seeds/pod produced, yield losses appear greater in the TRIF than in the BDS plants. Combining the number of flowers that might abort before pod and seed formation and the expected nematode intensity in a second generation, the magnitude of total yield loss in a field situation can be substantial especially if the first generation infection occurs in young seedlings.

In summary, nematode attack affected younger plants more than older plants, primarily due to the earlier effects of the nematode on APS rate and related physiological processes. These effects increase with level and duration of infection and as the
plants age they become less able to cope physiologically and accommodate a persistent parasite infection and, consequently, yield less than uninfected plants.

The data in this chapter are in press (see Melakeberhan et al., 1986 and see Appendix III).
CHAPTER VI

EFFECT OF MELOIDOGYNE INCognITA ON THE PHYSIOLOGY AND MINERAL NUTRIENT ELEMENTS OF PLANTS OF DIFFERENT AGE

INTRODUCTION

An adequate supply and distribution of nutrient elements enables normal plant growth. When plants are infected by nematodes, such as Meloidogyne, their nutrient and physiological status changes (eg. Bergeson, 1966). Chapter I reviewed the effect of nematodes on the nutrient concentration within plants and showed that changes are likely due to the type of host-parasite interaction and the time and intensity of infection. Moreover, it was shown that the way the data are expressed (eg. percentage or absolute values) is an important factor in identifying whether or not the nematode parasite influences a particular host parameter. Most earlier reports of nematode infections lacked the necessary supporting data on the physiological activities of the host to enable a complete understanding of the role of nutrient elements in the overall host physiology to be made.
My studies (chapter III) showed that at 3 wk after inoculation *M. incognita* causes an overall decrease in the content of calcium, potassium, manganese, iron, copper and zinc in the host plant. There was no significant effect of the nematode on the concentrations (per unit weight) of shoot copper and zinc, but that of shoot calcium and root potassium increased with increasing number of nematodes. It was concluded that changes in the concentration and uptake of these elements could interfere with chlorophyll synthesis and with APS rate and so result in reduced growth and crop yield of nematode infected plants. It is also believed that nutrient deficiency often has a synergistic rather than additive effect on the disease syndrome (Wallace, 1984).

My earlier studies (chapter III) included measurements of the APS rate and chlorophyll content, but the data were from samples taken only once during the period of infection. This chapter explores (1) the effect of a single generation of *M. incognita* on the nutrient concentration (ppm) and total nutrient content of French bean plants of different ages and at different stages of infection and (2) how these changes relate to certain host physiological processes.
MATERIALS AND METHODS

General experimental procedures and growth conditions were as described in chapter II. The methods for the determinations of CO₂ exchange rate, chlorophyll content and plant weight corresponding to the data reported in this chapter are given in chapters IV & V.

All experiments were done on French bean plants of different age within a single generation of M. incognita using destructive and non-destructive assays (see chapters IV & V).

For the destructive assay, one week-old plants were inoculated with 0 (control), 2000, 4000 or 8000 freshly hatched larvae, and the shoots and roots harvested at 1, 3, 8, 15, 22 and 27 d after inoculation. For the non-destructive assay, 3, 8, 11 and 13 day-old plants were inoculated with similar numbers of larvae, and the leaves, stems and roots were harvested separately at 27 or 28 d after inoculation. The separate plant tissue samples were dried, ground, made into pellets (approximately 140 mg/cm² thickness) and analyzed, using X-Ray energy spectroscopy (for 30 min), for potassium, calcium, manganese, iron, copper and zinc as described in chapter II. The error of variation of turning either side of the pellet to face the analysis beam was ± 4.1 % and that of the reproducibility of the concentration of the elements was ± 6.4 %.
Data were analysed using linear regression as outlined in chapter IV.

RESULTS

1 Destructive Assay

i) Concentration of elements

Except for an increase \((P \leq 0.01)\) in the concentration of root potassium with increasing number of nematodes 3 d after inoculation, there was no significant change in the other elements during the first week of infection (Fig. 26 - 28). The concentration of shoot and root potassium \((P \leq 0.01)\) at 15 d and shoot potassium \((P \leq 0.05)\) at 22 d following nematode inoculation was lower with increasing number of nematodes (Fig. 26). Root zinc at 15 d and shoot zinc at 22 d after inoculation decreased significantly \((P \leq 0.05)\) with increasing number of nematodes per plant (Fig. 26 & 28). At 27 d, however, the concentration of shoot potassium, manganese (Fig. 26 & 27), copper and zinc and root iron (Fig. 28) increased significantly \((P \leq 0.05)\) with the level of nematode infection.

The change in concentration over the duration of infection varied with the element, plant tissue and nematode treatment.
(Table 8). In the controls, the concentrations of potassium and zinc in the shoot and potassium and manganese in the roots increased \((P \leq 0.05)\) while shoot calcium and root iron decreased \((P \leq 0.05)\) with duration of infection. The shoot and root concentration of potassium and manganese increased \((P \leq 0.05)\) while that of calcium decreased \((P \leq 0.05)\) in all nematode treatments. Zinc in the shoots of all nematode treatments and copper in the roots of the two highest nematode treatments significantly \((P \leq 0.05)\) increased while root iron decreased \((P \leq 0.05)\) in all nematode treatments (Fig. 26 - 28 and Table 8).

Considering the importance of the potassium concentration in regulating the stomata and thereby the \(\text{CO}_2\) exchange rate, the APS rate of these plants was expressed on the basis of the concentration of shoot potassium. Generally, the APS rate significantly decreased \((P \leq 0.05)\) with an increasing level of nematode infection from one week after inoculation (Fig. 29).

ii) Total elemental content

On the first two sampling dates there was no significant change in nutrient content in any of the treatments. Except for root potassium and calcium at 8 d and total plant calcium at 22 d after inoculation, the content of potassium \((P \leq 0.01)\) and calcium \((P \leq 0.05)\) were significantly lower in all plant tissues with level of infection from one week after inoculation (Fig. 30). The manganese content in the shoot at 8 d, in all plant
NOTES FOR FIGURES 26 - 32

1) All figures show experimental data from the destructive assay.

2) Circled numbers refer to sampling dates after larval inoculation and the plant drawings to the stage of development at the time of larval inoculation.

Figure 26

The influence of *Meloidogyne incognita* on the concentration of potassium and calcium in shoots and roots of *Phaseolus vulgaris* over a 27 d period after nematode inoculation.
NUMBER OF NEMATODES RECOVERED /g FRESH ROOT WEIGHT
Figure 27

The influence of *Meloidogyne incognita* on the concentration of manganese and iron in shoots and roots of *Phaseolus vulgaris* over a 27 d period after nematode inoculation.
NUMBER OF NEMATODES RECOVERED
/g FRESH ROOT WEIGHT
Figure 28
The influence of *Meloidogyne incognita* on the concentration of copper and zinc in shoots and roots of *Phaseolus vulgaris* over a 27 d period after nematode inoculation.
NUMBER OF NEMATODES RECOVERED /g FRESH ROOT WEIGHT

Cu

- $y = -0.01x + 21$
- $r = -0.21$
- $y = -0.08x + 117$
- $r = 0.47$
- $y = 0.01x + 14$
- $r = 0.03$

Zn

- $y = 0.004x + 42$
- $r = 0.13$
- $y = -0.03x + 76$
- $r = 0.31$
- $y = 0.02x + 40$
- $r = 0.53$

---

ROOT

SHOOT
Table 8

Regression equations of the change in shoot and root concentration (ppm) of potassium, calcium, manganese, iron, copper and zinc in bean plants over a 27 d period after inoculation with four levels of *Meloidogyne incognita*

## ELEMENTAL CONCENTRATIONS

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<th>Ca</th>
<th>Mn</th>
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<th>Zn</th>
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</table>
Figure 29

The influence of *Meloidogyne incognita* on the photosynthetic rate of *Phaseolus vulgaris*, per unit shoot potassium basis over a 27 d period after nematode inoculation.
tissues at 15 d and in root and total plant content at 22 d and 27 d after inoculation was significantly \( (P \leq 0.05) \) lower with level of infection (Fig. 31). Iron was lower \( (P \leq 0.05) \) in the shoot at 8, 15 and 27 d after inoculation (Fig. 31). Zinc in the shoot at 8 d and all plant parts thereafter was significantly \( (P \leq 0.05) \) lower while copper was lower \( (P \leq 0.05) \) in all plant parts at 15 d and in roots at 22 d after inoculation (Fig. 32).

Generally, the overall content of the elements significantly \( (P \leq 0.05 - 0.01) \) increased with duration of infection and with decreasing number of nematodes. Exceptions were shoot and total plant iron and copper, which did not change significantly (Table 9).

2 Non-Destructive Assay

i) Concentration of elements

The first, second, third and fourth inoculation stages were done at 3, 8, 11 and 13 d after germination respectively and leaves, stems and roots harvested separately 4 wk later (Fig. 33 & 34). In the first inoculation (Fig. 33, left column), the concentration of potassium \( (P \leq 0.01) \) and zinc \( (P \leq 0.05) \) in all plant parts and calcium and copper \( (P \leq 0.05) \) in the roots was significantly lower with increasing nematode infection. There was no significant change in the concentration of manganese or iron in any of the plant parts.
The effect of *Meloidogyne incognita* on the content of potassium and calcium in shoots and roots of *Phaseolus vulgaris* plants over a 27 d period after nematode inoculation
NUMBER OF NEMATODES RECOVERED /g FRESH ROOT WEIGHT

133
Figure 31
The effect of *Meloidogyne incognita* on the content of manganese and iron in the shoots and roots of *Phaseolus vulgaris* plants over a 27 d period after nematode inoculation.
NUMBER OF NEMATODES RECOVERED
/g FRESH ROOT WEIGHT
Figure 32

The effect of *Meloidogyne incognita* on the content of copper and zinc in the shoots and roots of *Phaseolus vulgaris* plants over a 27 d period after nematode inoculation.
NUMBER OF NEMATODES RECOVERED /g FRESH ROOT WEIGHT
Table 9

Regression equations of the change in shoot, root and total plant content (µg) of potassium, calcium, manganese, iron, copper and zinc in bean plants over a 27 d period after inoculation with four levels of *Meloidogyne incognita*.

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<th>r</th>
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### ROOT ELEMENTAL CONTENT

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### ROOT ELEMENTAL CONTENT

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<th>Y</th>
<th>r</th>
<th>Mn</th>
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<td>0.61</td>
<td></td>
<td>0.3X + 13</td>
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<td>1.1X + 15</td>
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Table 9 Cont.

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<td>8000</td>
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In the second inoculation stage (Fig. 33, right column), the concentration of iron in the leaves \((P \leq 0.05)\), manganese in the stem \((P \leq 0.01)\) and zinc in the roots \((P \leq 0.05)\) increased while zinc in the stem \((P \leq 0.01)\) and calcium in the root \((P \leq 0.05)\) was significantly lower with the level of nematode infection. In the third inoculation stage (Fig. 34, left column), the concentration of potassium in the leaves \((P \leq 0.01)\), stems \((P \leq 0.001)\) and roots \((P \leq 0.001)\), zinc in the stem \((P \leq 0.01)\) and manganese in the roots \((P \leq 0.01)\) was significantly lower as the number of nematodes increased while stem and leaf calcium \((P \leq 0.01)\) increased. In the last inoculation stage (Fig. 34, right column), the concentration of potassium in the leaves \((P \leq 0.05)\) and manganese in the roots were significantly lower \((P \leq 0.01)\). However, calcium in all plant parts, manganese, iron and copper in the stems \((P \leq 0.05)\) and iron and zinc in the roots increased \((P \leq 0.05)\) significantly with increasing inoculum level.
NOTES FOR FIGURES 33 & 36:

1) All figures are from the non-destructive assay experiments.
2) Plant drawings refer to stage of plant development at the time of larval inoculation and circled numbers to the time of harvest.
3) Unless specified otherwise, the scale on both columns of each figure are the same.

Figure 33
The change in concentration of potassium, calcium, manganese, iron, copper and zinc in leaves, stems and roots of *Meloidogyne incognita* infected bean plants at 27 (left) and 28 (right) d after nematode inoculation. Plants on the left side were inoculated at 3 d and those on the right at 7 d after germination.
Inoculum level recovered / g fresh root weight

Number of nematodes

Inoculum level

Recoverd / g fresh root weight

Number of nematodes

Leaf

Stem

Root

 elemental concentration (ppm)

K (x 10^3)

Ca (x 10^3)

Mn (x 10^3)

Fe (x 10^3)

Cu

Zn

0 4000 8000

0 400 800
Figure 34
The change in concentration of potassium, calcium, manganese, iron, copper and zinc in leaves, stems and roots of *Meloidogyne incognita* infected bean plants at 28 d after nematode inoculation. Plants on the left side were inoculated at 11 d and those on the right at 13 d after germination.
ii) Total elemental content

At the first inoculation stage (Fig. 35, left column), the content of potassium, copper and zinc in all plant tissues, calcium in the leaves and roots and manganese in the shoot were significantly lower ($P \leq 0.001 - 0.05$) with increasing level of nematode infection. Iron content was significantly ($P \leq 0.05$) lower with level of infection in only the stems. In the second inoculation stage (Fig. 35, right column), the content of potassium and zinc in the shoot, manganese and iron in the leaves ($P \leq 0.05$) and calcium in the leaves and roots ($P \leq 0.01$) were significantly lower with increasing level of infection. Copper did not significantly change in any of the plant tissues. In the third inoculation stage (Fig. 36, left column), the content of potassium and manganese in all plant tissues, calcium and zinc in the shoot and copper in the stems were significantly ($P \leq 0.05$) lower with increasing number of nematodes. Iron did not significantly change. In the fourth inoculation stage (Fig. 36, right column), the content of potassium in the shoot, copper and zinc in the stem and manganese in the root were significantly ($P \leq 0.05$) lower with increasing level of infection. However, the content of calcium and iron in the stems and iron and zinc in the roots increased ($P \leq 0.05$) with level of infection. In most cases, degree of decline in elemental content was more on the first inoculation stage (Fig. 35 & 36).
Figure 35
The influence of *Meloidogyne incognita* on the total elemental content of potassium, calcium, manganese, iron, copper and zinc in leaves, stems and roots of *Phaseolus vulgaris* plants at 27 (left) and 28 (right) d after nematode inoculation. Inoculation dates were as in Fig. 33.
Figure 36

The influence of *Meloidogyne incognita* on the content of potassium, calcium, manganese, iron, copper and zinc on leaves, stems and roots of *Phaseolus vulgaris* plants at 28 d after nematode inoculation. Inoculation stages were as in Fig. 34.
LEAF ------ STEM ------ ROOT

NEMATODE INOCULUM
DISCUSSION

The collection of data at the termination of an experiment does not provide the full spectrum of effects over the life cycle of the nematode parasite or the development of the host. This study monitored the effect of a single generation of *M. incognita* at intervals on the growth of the plant. It does so by showing the changes in both the concentration and total content of different elements for plants inoculated at different ages and establishes a relationship between the nutrient status in the plant and some physiological processes, such as photosynthetic rate, that affect crop yield.

The total content of the elements was lower with increasing inoculum level from one week after inoculation. This corresponds with the plant dry weight (chapters IV & V) and supports the observations of Price et al. (1982) and Price & Sanderson (1984) on *Heterodera avenae* infected barley plants that lower nutrient contents in infected plants is due to the smaller plant biomass. The important factor, however, is the relative change in concentration of elements. Although there is no clear consensus concerning the mechanism of solute transport into and within the root that is applicable to all plants and solutes (Loughman, 1981), copper, iron and zinc are considered to be slowly absorbed compared with manganese, calcium and potassium.
The latter two elements are translocated via the apoplastic and symplastic routes, respectively (Clarkson, 1984).

However, in the destructive assay, the nutrient concentration did not change for over a week following nematode inoculation. This corresponds with the decline in APS rate reported in chapter IV. The concentration of iron, copper and manganese did not show any clear trend in the different parts of the bean plants over the life cycle of the nematode which suggests that the nematode does not affect them or the effect of the nematode on these elements is variable and probably is influenced by unidentified factors. Overall, calcium tended to increase and potassium and zinc showed a more consistent decrease with level of infection except for the destructive and the second inoculation stages of the non-destructive assays. These changes in concentration, how little or statistically insignificant they may be, could have an important effect in altering the host physiology.

Although the concentration of calcium decreased with duration of infection in the destructive assay, it increased with level of nematode infection at the end of most of the non-destructive assay experiments. This increased calcium concentration in the shoot could be explained as follows: First, being an easily absorbed element calcium is translocated fast to the shoot but, being less mobile once in the shoot, its concentration therein increases (Ayres, 1984 a). Second, at
advanced stages of nematode infection, chlorosis and abscission of the leaves increases (see chapter IV) which could result in the disintegration of cellular structures. Thus, the higher calcium concentration may indicate that it is being used for linking and modifying cell wall structures (McEuen et al., 1981; Clarkson, 1984) so that the plant maintains some level of photosynthesis. The increased calcium concentration could also have some disadvantages to the host. It is widely claimed that an increase in the concentration of calcium in the shoots of healthy bean plants (Biddulph et al., 1959) and other plants (Clarkson, 1984) or nematode infected potato plants (Evans et al., 1982) correlates with a high rate of translocation. Thus, the increased concentration of calcium in nematode infected plants suggests direct or indirect damage to the plant by the nematode (Evans & Franco, 1979; Evans et al., 1982). High calcium concentration in shoots is known to delay fruit maturity (Ferguson, 1984) and this could be one of the reasons for the delay in flowering that was observed in nematode infected plants in the previous three chapters.

Potassium has a wide range of biological activity including osmoregulation of the guard cells and, thereby, could affect the CO₂ exchange rate (Outlaw, 1983; Robinson & Preiss, 1985). Hence, the lower potassium concentration in the shoots and leaves of nematode infected plants with increasing numbers of nematode usually appears to correspond with increased root-galling, increased giant cell size and metabolic activity.

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(Bird, 1979 & see discussion chapter IV). This indicates that the uptake and/or transport of potassium may have been impeded by the deformation of the vascular tissues (Bergeson, 1966; Paulson & Webster, 1970; Wallace, 1974; Meon et al., 1978). Like Caenorhabditis elegans (Lu et al., 1983), M. incognita may have utilized potassium and other elements for its own growth and thereby decreased the available supply to and concentration in the shoot as shown by the correlation with decreased APS rate (Fig. 29).

Dijkshoorn et al. (1968) in rye grass and Ben-Zioni et al. (1971) in tobacco have demonstrated a recirculation of potassium in response to a change in balance of anions between plant shoots and roots. There was a slight increase in concentration of potassium at the harvest of the second inoculation stage together with a slightly better APS rate, compared with the previous sampling date. This suggests that the potassium may have been used for improved osmoregulation of the guard cells and/or as a balancing cation for other insufficient cations needed for the transport of organic anions such as photoassimilates to the roots. Furthermore, the slight change of trend in the concentration of potassium may explain the speculations regarding stimulatory and inhibitory processes of photosynthesis that exist in host-nematode interactions (Wallace, 1971 & 1974; see chapter V).

The interaction of calcium and potassium at the absorption, translocation and/or metabolism level is important. It is known
that cations exchange reversibly with identical or different cations dissolved in the soil solution (Loughman, 1981). Potassium, therefore, can be replaced by calcium in such solutions (Torimitsu et al., 1985; Salisbury & Ross, 1978) and the opposite trend of the data on these two elements suggest that *M. incognita* may be altering the balance of these elements. This further indicates that the decreasing and/or increasing trends in the concentration of the nutrient elements could have an important effect on the overall physiology of nematode infected plants. The fact that a range of elements are affected may be one reason why the symptoms observed in these nematode infected plants (see chapters IV & V) indicated more of a general than a specific effect. Associated with the symptoms observed in the previous chapters and the various physiological changes described above are the influences of the nematode induced changes at the infection site (see General Discussion, chapter VIII) and the translocation factor(s) (Fattah & Webster, 1984).

In summary, the data indicate that among the first effects of the nematode on host physiology is the change in concentration of the nutrient elements in the plant perhaps through the effect on plant growth hormones. These changes in nutrient concentration alter host metabolism and contribute directly (lack of or not in the right form) or indirectly (by affecting other processes) to the chlorosis and premature leaf abscission of infected plants. These effects on the host
increase with level and duration of infection, and along with changes in other physiological processes such as photosynthesis, appear to be the main cause for a lower yield in nematode infected plants. While I recognize the importance of biological interaction of the different elements to influence host growth and also that only a few of them were determined. Of those measured, the effect of potassium seems to be the most important in its effect on photosynthesis either by affecting the CO₂ uptake (see above) or altering other physiological processes such as osmotic potential.
INTRODUCTION

The role of potassium in osmoregulation of stomata and its influence on CO₂ exchange rate is well documented (see Salisbury & Ross, 1978). When external stress-inducing factors of either a parasitic or non-parasitic nature are involved, potassium content in the plant decreases and this appears to have far reaching consequences. Many studies have shown that potassium stress can either be reversed or diminished by foliar or root application of potassium and/or with other compounds (e.g. Swietlik et al., 1982 a, b & c; Geiger & Conti, 1983; Torimitsu et al., 1985). In nematode infected plants, however, this phenomenon has received very little attention.

Oteifa (1952) found no correlation between growth and high potassium application in heavily infected lima bean plants and at low nematode treatment there was almost a normal growth. Trudgill (1980) reported that increased fertilization of Globodera rostochiensis infected potato plants did not greatly
increase nutrient uptake, especially of phosphorus and potassium, or growth and yield of the plants. However, percent potassium in leaf dry weight was found to be closely correlated with growth and final yield. No study involving nematode-infected plants to date has attempted to correlate changes in the status of potassium with CO₂ exchange rate and seed yield.

Chapters III - V described a trend of decreasing APS rate with increasing level and duration of nematode infection. In chapter VI, potassium, among other elements, was lower in nematode infected plant leaves and shoots as the intensity of nematode infection increased. Furthermore, the APS rate based on shoot potassium concentration decreased with increasing nematode infection from one week after inoculation. This led to the conclusion that *M. incognita* may decrease the APS rate through decreasing levels of potassium in the leaves of nematode infected plants. Thus, indicating that potassium might be one of the root-derived factors that affect photosynthesis.

Therefore, the objective of this chapter was to test the hypothesis that 'root-derived factors affected photosynthesis' (Loveys & Bird, 1973) by applying different amounts of potassium to the soil and determining the influence of this treatment on the CO₂ exchange rate, growth, nutrient status and yield of *M. incognita* infected *P. vulgaris* plants.
MATERIALS AND METHODS

Prior to inoculation with nematodes, five approximately 350 g soil samples of the soil to be used in the experiment were analyzed for available nutrient concentration, conductance and pH status at the soils laboratory of the British Columbia Ministry of Agriculture and Fisheries, Kelowna. The concentrations of nitrate, phosphate, potassium, calcium, sodium, magnesium, copper, iron, manganese and zinc were determined using Inductively Coupled Plasma Atomic Emission Spectrometry (Church, 1981) and that of boron using the azomethine method (Upor et al. (1985). The concentration of nitrate was high while those of calcium and sodium were slightly lower (Table 10) compared with Agricultural soils. The concentration of each of the other elements were considered normal.

Five days after germination (0 time), the CO₂ exchange rate and leaf area of twenty seedlings was measured and individual plants were then randomly selected to receive one of the following treatments:

ONem1K = Hoagland solution (HS) without nematodes (control),
Nem0K = 4000 larvae + HS - potassium,
Nem1K = 4000 larvae + HS,
Nem2K = 4000 larvae + HS + double strength potassium and
Nem4K = 4000 larvae + HS + quadruple strength potassium.
Table 10

Soil nutrient concentration (μg/ml), conductance (dS/m) and pH of the mean of five samples before potassium treatment and nematode inoculation.

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<th>Standard Deviation</th>
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<td>Na</td>
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<td>B</td>
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<td>Fe</td>
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<td>Mn</td>
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</tbody>
</table>

Conductance 1.19 0.14
pH 4.62 0.14
Potassium in the Hoagland solution, and when added as a supplement, it was applied in the form of potassium nitrate.

All plants received Hoagland solution without potassium once before nematode inoculation and initiation of the treatment.

In this experiment, each set of four plants assigned to a treatment were inoculated one week after germination, arranged randomly in a growth chamber and watered with tap water or their particular nutrient treatment on alternate days.

The APS and dark respiration rates (at 22.5 ± 1 °C; 750 \( \mu \text{E.m}^{-2}.\text{s}^{-1} \) and air flow rate of 4.5 l.min\(^{-1}\)), leaf area measurements and the numbers of buds, flowers and/or pods were taken 0 time (which was 5 d after germination and 2 d before inoculation), and at 3, 7, 14 and 21 d after inoculation. At 28 d after inoculation, dry weights of leaves, stems and roots and numbers of pods, seeds and seeds/pod were determined.

Pellets of approximately 140 mg/cm\(^2\) in thickness for samples from leaves, stems and roots of each plant were prepared for X-ray energy spectroscopy analysis of potassium, calcium, manganese, iron, copper and zinc, as described in chapter II. The reproducability error of the concentrations of the elements was ± 7.6 %.

At the end of the experiment the soil nutrient concentration, conductance and pH of each potted plant were analyzed as in the beginning of the experiment.
Data for each sampling date were analyzed using an analysis of variance and Newman-Kuel test. The changes in leaf area and APS and dark respiration rates for each treatment with duration of infection were analyzed using linear regression.

RESULTS

About 12 d after inoculation, chlorosis developed in the primary leaves of the potassium deficient treatment. By 3 wk after inoculation, these symptoms developed on all primary leaves of the Nem1K and that of Nem0K, 75% of the Nem2K and 25% of the Nem4K and the control plants. Abscission of the yellowed leaves occurred about 1 wk later. More galls were visible on the Nem0K treated plants than on the other nematode treatments.

There was no significant difference in total leaf area in any of the treatments except at the last sampling date when that of the Nem4K treated plants was significantly higher \((P \leq 0.05)\) than the Nem0K and Nem1K treatments (Table 11 a). The leaf area of all treatments increased \((P \leq 0.01)\) with duration of infection until leaf abscission (Table 11 a).

The APS rate of the Nem1K treated plants was significantly less than that of Nem0K treatment 3 d after inoculation (Table 11 b). From the second week after inoculation, however, the APS rate of all nematode infected plants generally decreased with decreasing levels of potassium.
NOTES ON TABLES 11 - 15

1) Treatment abbreviations refer to those listed in the methods.
2) Means followed by the same letters are not significantly different at \( P \leq 0.05 \) (n=4).

Table 11

Influence of potassium on (a) leaf area (dm\(^2\)) and (b) photosynthetic and (c) dark respiration rate (mg CO\(_2\). dm\(^{-2}\).hr\(^{-1}\)) of nematode infected bean plants over a 28 d period.

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<th>3</th>
<th>7</th>
<th>14</th>
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<td>b</td>
<td>c</td>
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<td></td>
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<td>0.47  1.22 1.57 2.19 2.28 1.56ab 0.27X + 0.62 0.67</td>
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<td>NemOK</td>
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<tr>
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<tr>
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<tr>
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<td>ONem1K 2.3 0.7b 0.8 0.6 0.9ab 1.5a -0.1X + 1.5 -0.27</td>
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<tr>
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<tr>
<td>Nem2K</td>
<td>2.4 1.3a 0.7 0.6 1.1 a 1.0bc -0.2X + 1.9 -0.62</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nem4K</td>
<td>2.1 1.2a 0.8 0.4 1.1 a 1.2ab -0.3X + 2.1 -0.55</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In most sampling dates, the APS rate of the control plants was significantly higher than all but the Nem4K (Table 11 b).

The dark respiration rate of all nematode infected plants was significantly higher ($P \leq 0.05$) than the uninfected controls at 3 d after inoculation (Table 11 c). Three weeks after inoculation, the dark respiration rate of the Nem2K and Nem4K treated plants was higher than that of Nem0K treated plants and at the last sampling date the respiration rate of the Nem0K was significantly lower than that of the ONem1K and Nem4K treatments. The APS rate of all treatments (Table 11 b) and dark respiration rate of all nematode treatments (Table 11 c) decreased ($P \leq 0.01$) with duration of infection.

The leaf dry weight of the ONem1K was significantly higher ($P \leq 0.05$) than all but the Nem4K while the Nem4K was higher than the Nem0K and Nem1K (Table 12 a). The stem, shoot and total plant weight of the uninfected controls was significantly higher ($P \leq 0.05$) than all but the Nem4K. None of the treatments differed significantly in root weight (Table 12 a). The number of buds and flowers of the ONem1K and Nem4K was significantly higher than for other treatments at 14 d and the ONem1K treatment significantly higher than for Nem0K and Nem1K at 21 d after inoculation (Table 12 b). At 28 d after inoculation, the numbers of pods and seeds of the uninfected controls was higher ($P \leq 0.05$) than all but the Nem4K treatment, and those of Nem4K from the Nem0K (Table 12 b).
Table 12

The effect of potassium treatment on (a) plant dry weight at 28 d and (b) yield components of *Meloidogyne incognita* infected *Phaseolus vulgaris* plants over 28 d after inoculation. (n=4).

PLANT DRY WEIGHT (g)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Leaf</th>
<th>Stem</th>
<th>Shoot</th>
<th>Root</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONem1K</td>
<td>0.87 a</td>
<td>0.60 a</td>
<td>1.46 a</td>
<td>0.39</td>
<td>1.85 a</td>
</tr>
<tr>
<td>Nem0K</td>
<td>0.41 c</td>
<td>0.30 b</td>
<td>0.70 b</td>
<td>0.30</td>
<td>1.00 b</td>
</tr>
<tr>
<td>Nem1K</td>
<td>0.35 c</td>
<td>0.37 b</td>
<td>0.72 b</td>
<td>0.30</td>
<td>1.02 b</td>
</tr>
<tr>
<td>Nem2K</td>
<td>0.58 bc</td>
<td>0.37 b</td>
<td>0.95 b</td>
<td>0.27</td>
<td>1.22 b</td>
</tr>
<tr>
<td>Nem4K</td>
<td>0.69 ab</td>
<td>0.46 ab</td>
<td>1.14 ab</td>
<td>0.29</td>
<td>1.43 ab</td>
</tr>
</tbody>
</table>

(b)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>BUDS and FLOWERS</th>
<th>PODS</th>
<th>SEEDS</th>
<th>SEEDS/POD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONem1K</td>
<td>12.50 a</td>
<td>11.00 a</td>
<td>6.00 a</td>
<td>28.50 a</td>
</tr>
<tr>
<td>Nem0K</td>
<td>9.50 b</td>
<td>8.00 b</td>
<td>3.00 c</td>
<td>11.25 c</td>
</tr>
<tr>
<td>Nem1K</td>
<td>9.25 b</td>
<td>7.50 b</td>
<td>4.00 bc</td>
<td>14.50 bc</td>
</tr>
<tr>
<td>Nem2K</td>
<td>8.75 b</td>
<td>8.75 ab</td>
<td>4.00 bc</td>
<td>16.25 b</td>
</tr>
<tr>
<td>Nem4K</td>
<td>12.00 a</td>
<td>10.25 ab</td>
<td>5.25 ab</td>
<td>23.25 ab</td>
</tr>
</tbody>
</table>
Table 13

The relationship between different levels of potassium treatment and the (a) leaf, (b) stem and (c) root elemental concentration (ppm) of *Meloidogyne incognita* infected bean plants. (n=4).

<table>
<thead>
<tr>
<th>TREATMENT ELEMENTS</th>
<th>ELEMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K</td>
</tr>
<tr>
<td>a</td>
<td>ONem1K</td>
</tr>
<tr>
<td></td>
<td>NemOK</td>
</tr>
<tr>
<td></td>
<td>Nem1K</td>
</tr>
<tr>
<td></td>
<td>Nem2K</td>
</tr>
<tr>
<td></td>
<td>Nem4K</td>
</tr>
<tr>
<td></td>
<td>ONem1K</td>
</tr>
<tr>
<td></td>
<td>NemOK</td>
</tr>
<tr>
<td></td>
<td>Nem1K</td>
</tr>
<tr>
<td></td>
<td>Nem2K</td>
</tr>
<tr>
<td></td>
<td>Nem4K</td>
</tr>
<tr>
<td>b</td>
<td>ONem1K</td>
</tr>
<tr>
<td></td>
<td>NemOK</td>
</tr>
<tr>
<td></td>
<td>Nem1K</td>
</tr>
<tr>
<td></td>
<td>Nem2K</td>
</tr>
<tr>
<td></td>
<td>Nem4K</td>
</tr>
</tbody>
</table>
The number of seeds/pod in the Nem1K was significantly lower ($P \leq 0.05$) than for ONem1K and Nem4K treatments.

In all treatments, the concentration of potassium in the leaves and stems (shoots) significantly increased while calcium significantly decreased ($P \leq 0.05$) between the Nem0K and Nem4K treatments (Table 13 a & b). The shoot manganese concentration of the uninfected controls and Nem4K was significantly ($P \leq 0.05$) lower than the rest of the treatments (Table 13 a & b). The shoot iron of the Nem0K was significantly ($P \leq 0.05$) higher than all other treatments (Table 13 a & b). In the Nem4K treatment, the leaf zinc concentration was significantly ($P \leq 0.05$) lower than that for other treatments while in the stem Nem0K it was significantly lower ($P \leq 0.05$) than in ONem1K. None of the elements in the roots changed significantly (Table 13 c). Generally, however, the potassium and calcium concentrations were higher in the shoots than the roots while manganese, iron and copper tended to be more concentrated in the roots than the shoots (Table 13).

The total content of potassium in the leaves and stems of nematode infected plants significantly increased ($P \leq 0.05$) with the level of potassium (Table 14 a & b). The potassium content in leaf and in the stem of the Nem4K treatment was significantly ($P \leq 0.05$) higher than that of the ONem1K (Table 14 a & b). The content of manganese and zinc in the leaves of the ONem1K and Nem4K were significantly higher than for Nem0K and Nem1K (Table 14 a).
Table 14
The effects of different levels of potassium treatments on the nutrient elemental content (mg) of (a) leaves, (b) stems and (c) roots of *Meloidogyne incognita* infected bean plants. (n=4).

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>ELEMENTS</th>
<th>K</th>
<th>Ca</th>
<th>Mn</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0Nem1K</td>
<td></td>
<td>65.45 b</td>
<td>1.913 a</td>
<td>1.285 a</td>
<td>0.705 b</td>
<td>0.031</td>
<td>0.059 a</td>
</tr>
<tr>
<td>NemOK</td>
<td></td>
<td>16.18 c</td>
<td>1.346 ab</td>
<td>0.851 b</td>
<td>1.484 a</td>
<td>0.012</td>
<td>0.026 b</td>
</tr>
<tr>
<td>Nem1K</td>
<td></td>
<td>23.88 c</td>
<td>1.061 b</td>
<td>0.773 b</td>
<td>0.664 b</td>
<td>0.009</td>
<td>0.024 b</td>
</tr>
<tr>
<td>Nem2K</td>
<td></td>
<td>65.68 b</td>
<td>1.341 ab</td>
<td>1.114 ab</td>
<td>1.463 a</td>
<td>0.014</td>
<td>0.038 ab</td>
</tr>
<tr>
<td>Nem4K</td>
<td></td>
<td>110.98 a</td>
<td>1.405 ab</td>
<td>1.071 ab</td>
<td>0.651 b</td>
<td>0.022</td>
<td>0.036 ab</td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0Nem1K</td>
<td></td>
<td>26.79 b</td>
<td>0.463</td>
<td>0.206</td>
<td>0.292</td>
<td>0.012</td>
<td>0.043 a</td>
</tr>
<tr>
<td>NemOK</td>
<td></td>
<td>9.54 c</td>
<td>0.406</td>
<td>0.195</td>
<td>0.273</td>
<td>0.005</td>
<td>0.015 c</td>
</tr>
<tr>
<td>Nem1K</td>
<td></td>
<td>15.22 c</td>
<td>0.483</td>
<td>0.243</td>
<td>0.210</td>
<td>0.005</td>
<td>0.023 bc</td>
</tr>
<tr>
<td>Nem2K</td>
<td></td>
<td>27.13 b</td>
<td>0.391</td>
<td>0.251</td>
<td>0.204</td>
<td>0.007</td>
<td>0.022 bc</td>
</tr>
<tr>
<td>Nem4K</td>
<td></td>
<td>47.15 a</td>
<td>0.473</td>
<td>0.183</td>
<td>0.197</td>
<td>0.005</td>
<td>0.032 b</td>
</tr>
<tr>
<td>c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0Nem1K</td>
<td></td>
<td>24.98</td>
<td>0.477</td>
<td>1.72</td>
<td>4.47</td>
<td>0.036</td>
<td>0.040</td>
</tr>
<tr>
<td>NemOK</td>
<td></td>
<td>12.92</td>
<td>0.256</td>
<td>0.98</td>
<td>3.46</td>
<td>0.021</td>
<td>0.032</td>
</tr>
<tr>
<td>Nem1K</td>
<td></td>
<td>14.49</td>
<td>0.341</td>
<td>1.13</td>
<td>3.42</td>
<td>0.023</td>
<td>0.031</td>
</tr>
<tr>
<td>Nem2K</td>
<td></td>
<td>20.09</td>
<td>0.307</td>
<td>1.44</td>
<td>2.74</td>
<td>0.019</td>
<td>0.034</td>
</tr>
<tr>
<td>Nem4K</td>
<td></td>
<td>14.13</td>
<td>0.255</td>
<td>1.16</td>
<td>2.47</td>
<td>0.018</td>
<td>0.034</td>
</tr>
</tbody>
</table>
The stem zinc content of the ONem1K was significantly ($P \leq 0.05$) more than for all nematode treatments (Table 14b). None of the elements significantly ($P \leq 0.05$) changed in content in the roots (Table 14c).

At the end of the experiment, there was no significant change in the amount of phosphate, sodium, boron, iron and zinc in the soil of any of the treatments (Table 15). The nitrate and potassium concentration and conductivity (salts) of soil from the Nem2K and Nem4K potassium treatments increased ($P \leq 0.05$) compared with those of the other treatments. The pH values of the soil decreased with nematode treatment and increasing potassium applications. Calcium and sulphate was significantly lower ($P \leq 0.05$) in the Nem0K and Nem1K compared with that of the uninfected controls and in the Nem4K, and manganese was significantly lower ($P \leq 0.05$) in the Nem2K compared with the Nem0K treatments (Table 15). Copper was significantly higher in the Nem0K and Nem1K treated soils than in soils from any other treatments (Table 15).
Table 15

Nutrient elemental concentration (µg/ml), conductance (dS/m) and pH status of soil at 28 d after potassium treatment was applied for *Meloidogyne incognita* infected bean plants. (n=4).

<table>
<thead>
<tr>
<th>ELEMENTS</th>
<th>TREATMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ONem1K</td>
</tr>
<tr>
<td>PO₄</td>
<td>119.50</td>
</tr>
<tr>
<td>Na</td>
<td>23.75</td>
</tr>
<tr>
<td>B</td>
<td>0.64</td>
</tr>
<tr>
<td>Fe</td>
<td>41.95</td>
</tr>
<tr>
<td>Zn</td>
<td>0.63</td>
</tr>
<tr>
<td>NO₃</td>
<td>90.25</td>
</tr>
<tr>
<td>K</td>
<td>135.50</td>
</tr>
<tr>
<td>Ca</td>
<td>326.25</td>
</tr>
<tr>
<td>Mg</td>
<td>53.75</td>
</tr>
<tr>
<td>SO₄</td>
<td>49.30</td>
</tr>
<tr>
<td>Cu</td>
<td>1.03</td>
</tr>
<tr>
<td>Mn</td>
<td>4.88</td>
</tr>
<tr>
<td>Conductance</td>
<td>1.00</td>
</tr>
<tr>
<td>pH</td>
<td>4.93</td>
</tr>
</tbody>
</table>

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DISCUSSION

Commercial fertilizer is commonly used for improving crop yield as 1) a supplement to the existing levels in the soil, 2) an addition to overcome nutrient deficiency and 3) to compensate for losses that result from pathogens such as nematodes (eg. Oteifa, 1952; Trudgill, 1980; Spiegel et al., 1982). The use of commercial fertilizer where a host-parasite is involved, however, could be better exploited if the effects of the pathogen on the host physiological processes that affect crop yield are understood. In the experiment reported here, the relationships between supplementary potassium treatment, CO₂ exchange rate and yield of M. incognita infected bean plants are shown. Overall, there was a significant positive correlation between high supplementary potassium, the photosynthetic rate and seed yield of nematode infected plants compared with the potassium deficient nematode treatments.

Compared with the uninfected controls, the APS rate did not significantly decrease with decreasing amount of potassium until 2 wk following nematode inoculation probably because the inoculum level was too low to express a change at an earlier sampling date. However, high supplementary potassium appeared to
increase dark respiration rates, particularly during maturation of the nematode, a time which corresponds with the host demand for more photosynthate to support the requirement of flower and seed production. The relationship between increased potassium concentration in the leaves and the CO$_2$ exchange rate, particularly APS rate, parallels the common phenomenon in healthy plants that receive external supplementary potassium (e.g. Geiger & Conti, 1983; Torimitsu et al., 1985). This relationship may be due to the intracellular accumulation of potassium in the leaves (Geiger & Conti, 1983) which could improve stomatal regulation in a manner favouring a higher CO$_2$ exchange rate (Outlaw, 1983; Robinson & Preiss, 1985). Potassium, therefore, could be one of the "root-derived (translocated) factors" (Loveys & Bird, 1973) that influence photosynthesis in nematode infected plants. In saying so, I recognize that the 'exact' effect of the nematode on photosynthesis is not clear and the chlorosis symptoms observed in this experiment resemble those caused by other non-nematode-induced stresses. The stresses could affect the light or dark reactions of photosynthesis with little effect on the stomata (Salisbury & Ross, 1978) and lead to similar symptoms. What my results show is a correlation between increased potassium concentration and APS rate which suggests that potassium may have lowered the water potential of the leaves. This increases the up-take of nutrient elements which otherwise would have been impeded (see below).
Associated with the increase in potassium concentration in the shoots of high supplementary potassium treatment is the change in concentration of the nutrient elements in the soil and the different plant parts. In this experiment, the concentration of potassium and of nitrate along with the salts increased while calcium, manganese and sulphate decreased with high supplementary potassium levels. It should be noted that as the increased potassium was added in the form of potassium nitrate, the nitrate component was also increased accordingly and may have a confounding effect on the results. These changes could affect the equilibrium in the soil which, in turn, could alter the uptake and/or metabolism of these elements within the plant (Brown, 1978).

At the osmotic level, bean (a glycophyte plant), (Meiri & Polyjkoff-Mayber, 1970; Nassery & Jones, 1976) and M. incognita (Viglierchio et al., 1969; Edongali & Ferris, 1982; Edongali et al., 1982) are both sensitive to high salt concentrations. Thus, the increased galling in the Nem0K compared with the Nem4K treatments indicate that the nematode's ability to invade and/or induce galling may have been impaired by high salt concentration in the soil. Since the number of nematodes in the roots was not estimated, this point could not be clarified. However, with the parallel increase of salt concentration and supplementary potassium levels in the soil, one could have expected salinity problems to affect photosynthesis (Wignarajah et al., 1975 a & b; Mawson & Colman, 1983; Seemann & Critchley, 1985) and thereby
to decrease plant growth (Brown, 1978; Lahaye & Epstein, 1971; Maas & Nieman, 1978). In this study, however, such a relationship was not the case probably because of the inverse relationship between the increased salt concentration and pH values. However, the high nitrate, sulphate and possibly other undetermined elements such as chlorine in the soil in the high supplementary potassium treatments show that they might be responsible for the decrease in pH values and thereby offset the salinity problem.

The increased potassium concentration in the shoots with increasing levels of supplementary potassium in the soil shows that the more available potassium there is the more the plant absorbs. Furthermore, the significant accumulation of potassium in the shoots may enhance its subsequent recirculation to the sinks (flowers and roots) (Geiger & Conti, 1983), and probably enhance the movement of other ions to maintain the anion and cation equilibrium (Ayres, 1984 a). The inverse relationship between calcium concentration in the leaves and/or stems of nematode infected plants and increasing levels of supplementary potassium supports the notion that the accumulation of calcium may be of an advantage or a disadvantage to the host (see Discussion chapter VI). It also indicates that calcium may be affected by potassium triggered conditions in the rhizosphere (Johansen et al., 1968). The distribution of elements within the plant generally showed that potassium, calcium and iron were concentrated mainly in the shoots whereas manganese, copper and
zinc were mainly in the roots. This unbalanced distribution could significantly affect the host metabolism in such a way that senescence is enhanced and this decreases host productivity.

In summary, the degree of chlorosis of nematode infected plants in the potassium deficient treatments agrees with earlier statements (chapter III - VI) and indicates that there may be some "root-derived" toxicity factor involved. The results also support the claim (chapter VI) that nutrient elements might be quantitatively or qualitatively the first to be affected as a result of nematode invasion of the roots. This influences host metabolism and other aspects of the host physiology (see also chapter VI). Moreover, the results support earlier statements (chapter VI) that *M. incognita* decreases APS rate by decreasing potassium levels in the plant. This can be reversed or improved by high supplementary potassium application, up to four times the normal rate, which increases the concentration of potassium (along with other elements) in the shoots. This increases the APS rate which, in turn, increases growth and yield and is evidence of potassium being one of the "root-derived factors" that affect photosynthesis and related processes. In practical terms, the results suggest that yield could be improved by supplementary potassium, but this requires further analysis of the optimum level of potassium without adversely changing the cation and anion balance in the soil and plant.
CHAPTER VIII
GENERAL DISCUSSION AND CONCLUSIONS

Crop loss assessment is emerging as a specialized field of technology for crop protection strategies, insurance adjustments and marketing predictions (Russell, 1981; Loomis & Adams, 1983). Precise crop loss assessment requires proper identification and quantification of the factors influencing the host (Main, 1983) and an understanding of the mechanisms by which these factors affect their hosts (Dowler & Van Gundy, 1984). However, the precision of crop loss assessment in nematode-host interaction has been incomplete by being from multiple generation studies or lacking physiologically based data. My study is the first of its kind to include a broad range of physiological processes and morphological parameters and to show how these parameters are affected by a single generation of the nematode with resultant loss in host productivity. This holistic approach provides a good perspective and opportunity to better understand the mechanisms by which nematodes induce loss of yield.

As the host physiological response and yield loss varies with the number of nematodes in the roots, it is important to determine good estimates of the nematode population (Barker & Campbell, 1981; Ferris, 1984). The collecting of root samples, however, is subject to error (Barker & Campbell, 1981) and sometimes misleading estimates of the nematode population may be
made if, for instance, the data are not based on good sampling methods (see chapter V). In my experiments, the number of nematodes recovered is proportional to the inoculum level (see chapters III & IV) and this likely is due to the particular range of inoculum size utilized as otherwise a sigmoid relationship would have been more likely (Seinhorst, 1981 a & b). Furthermore, all of the measured physiological, morphological and yield parameters showed a similar trend and level of significance when expressed on the basis of either inoculum level or number of nematodes recovered from the roots.

In order to understand the growth and yield loss caused by nematode infection, the biological processes involved in both healthy and infected plants must be known. If one analyzes the biological systems in hierarchic levels from molecules to ecosystems, the behaviour observed at the cellular level is explained by the underlying molecular events, eg. enzymatic or hormonal control, or by factors imposed from higher levels eg. water status (Loomis & Adams, 1983). Moreover, depending upon the nematode's mode of infection in the plant, the morphological changes in the host or seed losses may not be a result of the direct effect of nematodes on those tissues or physiological processes occurring in the foliage but a result of events that occur at the infection site. As outlined by Dropkin (1980), plant-parasitic nematodes can be divided into three distinct groups according to the changes they induce at their feeding sites. These are: (1) destructive changes—where the host cells
are destroyed eg. many of the migratory endo- and ecto-parasites, (2) adaptive changes- where the host cells modify their behaviour eg. *Tyl enchulus semipenetrans* and (3) neoplastic changes-where the host cells undergo new growth eg. *Meloidogyne* spp. Depending on the number of nematodes infecting the plant, one can expect that the nematode-induced physiological changes and yield loss will generally increase with the degree of sophistication of the nematode's mode of feeding (Dropkin, 1980).

Photosynthesis is one of the most crucial physiological processes that influence plant growth and crop yield. Photosynthesis and host productivity are affected by many factors including stress from pathogen infection, the process of CO₂ exchange rate, water relations, chlorophyll content, nutrient uptake and metabolism, translocation and the status of plant growth regulators. As indicated earlier, however, the influence of nematodes on photosynthesis has received very little attention until now (Loveys & Bird, 1973; Wallace, 1974; Franco, 1980) and certainly not sufficient to help understand the mechanisms by which particular factors affect it. Depending on host age at the time of inoculation, my study has shown in general that the APS rate decreases as early as 3 d after nematode inoculation on leaf area basis and at 8 d on a total chlorophyll, total shoot nitrogen or shoot potassium concentration basis. This reflects a decline in chlorophyll content or activity and nutritional elements associated with
chlorosis and abscission of the primary leaves. The severity of the decline in APS rate generally increased with level and duration of nematode infection and resulted in a corresponding lower yield with the increasing levels of nematode infection.

All other factors constant, plant productivity is dependent on the amount of CO$_2$ fixation and the subsequent partitioning of the photosynthate material (Thorpe & Lang, 1983) which, in turn, are affected by the rates of dark respiration and photorespiration (Butler, 1978; Wallsgrove et al., 1983) and the source and sink factors (Herold, 1980; Whipps & Lewis, 1981). All of these processes are influenced by pathogens (Daly, 1976; Kosuge & Kimpel, 1981) and/or physio-chemical and environmental factors (Krampitz & Fock, 1984; Krampitz et al., 1984).

In most plant-pathogen interactions, other than those involving nematodes, it is believed that dark respiration rate is increased almost invariably by infection (Daly, 1976). In my experiments, dark respiration was not consistently influenced by either level or duration of nematode infection and this conforms with earlier observations (Bird & Miller, 1962; Professor K. R. Barker, personal communication). Since root respiration was not measured, however, it is not known what effect *M. incognita* may have had on the respiration rate of the whole bean plant as distinct from the shoots alone. Moreover, the C$_3$ (Calvin cycle) plants, to which *Phaseolus* spp. belongs, have a high photorespiration rate (Bykov et al., 1981) and photorespiration is increased by rust fungi infection (eg. Raggi, 1978). It is
possible that *M. incognita* may have increased this wasteful process in the bean plant and adversely affected host productivity.

The products of photosynthesis are used for maintenance and growth of the plant (Daly, 1976; Whipps & Lewis, 1981) through the activity of the primary (cytoplasm) and secondary sinks (flowering and storage, and active growth of root and shoot tissues) (Sinclair & de Wit, 1975; Herold, 1980). The competition and demand for photosynthates by the components of these two sinks likely alters the growth and productivity of the plant (Small & Leonard, 1969; Thorpe & Lang, 1983). For example, sucrose synthesis, among other processes, occurs in the cytoplasm and consumes a major fraction of the photosynthates (Bird *et al.*, 1974). The translocation of sucrose, the major form of sugar translocated (Salisbury & Ross, 1978), will likely be more influenced by sink demands when the host plant is infected with root-knot nematodes which also have a high demand for photosynthetic material (Bird & Loveys, 1975).

The demand for photosynthates by secondary sinks can have significant consequences on plant yield (Sinclair & de Witt, 1975). Photosynthesis is an important factor during the storage and grain filling phases (Evans, 1975). However, storage capacity is dependent on the conditions prior to flowering that generate reserves which may be mobilized during the storage phase (Evans, 1975). Under my experimental conditions, photosynthesis was decreased by nematode infection before and/or
at the flowering stage. This could be one reason for the decreased yield capacity and/or delayed maturity of the nematode infected plants (see chapters III - VII). Furthermore, there may have been competition for photosynthetic material between the nematode infected roots and the flowers. In contrast to the results of Wallace (1974), Bird & Loveys (1975) and McClure (1977) showed that *Meloidogyne* infected tomato root galls accumulated high concentrations of $^{14}$CO$_2$ compared with that of healthy root tissues, and concluded that the nematode was acting as a metabolic sink. In my opinion, these differences in results might be due to differences in their respective experimental design. However, being an obligate parasite, *M. incognita* is likely to be utilizing photosynthates for its own development and/or the materials may have leaked out of the plant cells into the soil along with other metabolites (eg. Van Gundy *et al.*, 1977). I suggest this because the yield components and root mass (except for the occasional components of the data that were not significant) are consistently lower in nematode infected plants as compared with the controls.

Pathogen-induced changes (Duniway, 1973; Ayres; 1984 a) and particularly those caused by root infecting nematodes can alter host water relations (eg. Odihirin, 1970; Seinhorst, 1981 a & b; Evans, 1982). Schoeneweiss (1978) summarised effects of lack of water as follows: (1) Decreased photosynthesis due to stomatal closure, decreased CO$_2$ diffusion and decreased chloroplast activity. (2) Increase in some hydrolytic enzymes and a decrease
in other enzymes. (3) Initial increase followed by a decrease in respiration. (4) An increase in abscisic acid and decrease in cytokinins. (5) Reduction of protein synthesis and decomposition of proteins and nucleic acids. (6) Accumulation of proline and sugars and (7) general decrease in nutrient uptake, particularly potassium and phosphorus. It is difficult to identify which of these factors is acting at any one time in the physiological process (Schoeneweiss, 1978) and it is further complicated by the introduction of pathogens. Furthermore, the resemblance of chlorosis and leaf abscission symptoms that were observed in my study compared with other non-nematode-induced stress factors suggest that they may have similar effects on the photosynthetic process.

As stated in chapter I, previous studies generally indicate that the water absorption capacity of the host plant is decreased by nematode infection of the roots, and consequently this affects transpiration and photosynthesis. The effect of nematodes on transpiration and on water-use efficiency vary with the host-parasite interaction and time of measurement after inoculation (Odihirin, 1970; Elkins et al., 1979). However, the effect of nematodes on transpiration and photosynthesis seems unclear and is compounded by the effect of nematodes on the host nutrient status, especially that of calcium and potassium. The question then arises as to what the relationships are between photosynthesis, transpiration and the concentrations of calcium and potassium?
Divalent cations, such as calcium, are dominant over monovalent cations in the apoplastic route (Nissen, 1974; Pitman, 1977). Hence, high calcium concentration in the shoots of either healthy plants (e.g. Biddulph et al., 1959; Ayres, 1984a; Clarkson, 1984), those infected with fungi (Ahmed et al., 1982) or nematodes (Evans et al., 1982) would be an indicator of the total volume of transpiration. The APS rate (Goudriaan & van Laar, 1978) and the leaf (shoot) concentration of potassium (Salisbury & Ross, 1978) are indirect ways of describing stomatal conductivity. Thus, the decreased APS rate and potassium concentration indicates that transpiration probably decreased. Hence, the increased calcium concentration in the shoots of nematode infected plants indicates one and/or both of the following: (1) its limited mobility once in the shoot tissues (see discussion of chapter VI & VII), (2) the effect of *M. incognita* on transpiration of bean plants may be variable but not detected here on the time scale used for sampling.

In a disease syndrome, the effect of decreased nutrient content (concentration) is not additive but synergistic (Wallace, 1984). In considering nutrient elements it is important to note significant as well as insignificant changes in the concentration of the different elements in the different plant tissues. Statistically insignificant changes tend to be overlooked. Physiologically, however, a slight deviation from the norm of a particular process caused by a highly specialised parasite such as *M. incognita* could have major consequences in
changing other host physiological processes. Moreover, photosynthesis and chlorophyll, crucial components of the photo-assimilation process, are very sensitive to the plant's nutrient status.

Loveys & Bird (1973) hypothesized that a "reduced supply of root-derived factors influenced photosynthesis of nematode infected plants" but the hypothesis was not tested. As the term 'root-derived factors' from nematode parasitized roots is too general, I would like to split such factors into the following categories: (1) the root originating factors, namely, root products eg. phytohormones, (2) the nematode originating factors, namely, nematode products eg. exudates and (3) the translocated factors, namely, nutrients and water.

The increased concentration of foliar potassium, rate of photosynthesis and yield with increasing levels of supplementary potassium to nematode infected plants, compared with those receiving little or no supplementary potassium, is direct evidence in support of the hypothesis that root-derived factors affect photosynthesis. Potassium is thereby identified as one of the root-derived translocated factors. Although the exact mechanism by which potassium is improving the host capacity is not known, the increased concentration of potassium may satisfy the deficit, due to nematode uptake (see chapter VI), root growth (Trudgill, 1980) or due to leakage so as to balance some anions in the rhizosphere, and continue to increase photosynthesis and yield.
Plant growth hormones, such as cytokinins and gibberellins, are manufactured in the roots (Itai & Vaadia, 1971; Newman & Stein, 1983; Scott & Horgan, 1984) and the amount produced in nematode-free bean plants is proportional to the size of the root system (Carmi & Heuer, 1981). Hence, the smaller root weight of the nematode infected plants suggests that the production of these hormones may have been decreased. These hormones are translocated to the shoots and utilized in the control of cell elongation and cell division (Kefeli, 1978), and for the translocation and distribution of photo-assimilates (Seth & Wareing, 1967; Small & Leonard, 1969; Thorpe & Lang, 1983) and nutrient elements (see later). This further shows their influence on shoot growth (Carmi & Heuer, 1981) and foliar symptom expression.

Cytokinins are particularly important in relation to leaf senescence (e.g. Woolhouse, 1967 & 1974; Thomas & Staddart, 1980). In my experiments, root weight and most elements of nematode infected plants were lower one week after nematode inoculation compared with the controls. This was followed by leaf abscission, a change in nutrient concentration and a decline in chlorophyll content. Besides decreased production due to smaller root weight, it is possible that the translocation to and the activity of these hormones in the shoots was altered by the nematode. Under these circumstances, the host physiology changes in such a way that chlorophyll content and/or activity declines (Maunders et al., 1983), resulting in increased leaf
senescence (Chatterjee et al., 1965; Newman & Stein, 1983) and a decline in photosynthesis, all of which were evident in the present study. Moreover, cytokinins and ethylene have opposite roles in controlling growth (Thomas & Staddart, 1980). Glazer et al. (1983 & 1984) showed an increase in ethylene about 2 wk after inoculating tomato plants with M. javanica which corresponds with the onset of senescence that was observed in my experiments. This indicates that leaf senescence in nematode infected bean plants may also be associated with a decline in cytokinin and an increase in ethylene in the shoots. The nematode's ability to increase the concentration of plant growth regulators, such as cytokinins in and around root galls could decrease their concentration in the shoots (Dimalla & Van Staden, 1977; Van Staden & Dimalla, 1977) and result in the above discussed symptoms. The concentration of shoot cytokinin could vary with the developmental stage of the nematode (Davey & Van Staden, 1976; Bird et al., 1980). This may as a corollary be a factor that influences the change in concentration and distribution of nutrient elements within the plant (Mauk & Nooden, 1983; Newman & Stein, 1983). The relationship between the nutrient elements and growth hormones in relation to senescence suggests that the nematode may be affecting what the roots produced which, in turn, affects the uptake rate and translocation of nutrient elements and the foliage physiology of nematode infected plants.
The overall effect of these physiological processes is manifested in the morphological appearance of the host plant. The primary targets of the physiological processes influenced by the nematode are the status of water, nutrient elements, phytohormones, CO₂ exchange rate and chlorophyll content and/or activity. The phytohormones and water relations were not measured in this study and so reference to them is based on data available from the literature. Galling is initiated within a few hours of nematode inoculation (Paulson & Webster, 1970) and before the nematodes establish themselves in the vascular tissue (Bird, 1979). Hence, it is likely that the plant growth hormones are one of the first substances to be altered by nematode attack. This would in turn modify the uptake and/or translocation of water and nutrient elements and, subsequently, affect the CO₂ exchange rate (particularly APS rate), chlorophyll content and/or photosynthate production in the leaves. This decrease in foliar physiological productivity diminishes the metabolite supply to the roots which have lower than optimum efficiency and are further influenced by the persistent nematode infection on the roots. The circle of changed physiological processes is thereby completed and at the same time cell division and expansion is slowed. Eventually, this affects the host morphology resulting in a smaller leaf area which directly affects the photosynthetic capacity of the infected plants and so compounds the primary effect of the nematodes on photosynthesis. The overall, smaller plant biomass
and slower development leads to diminished yield as fewer seeds are produced compared with control plants.

In summary, the thesis has addressed the problems of precise crop loss assessment of *M. incognita* infected bean plants by studying the physiological processes and morphological characteristics associated with crop yield. For the first time, this was done by using a single generation of the nematode infection on a *P. vulgaris* cultivar that produces seeds within 5 wk of germination. Specifically, the research identifies the following points:

(1) The major physiological processes that are affected by the nematode are APS rate (on a leaf area, total chlorophyll, total shoot nitrogen or shoot potassium concentration basis), chlorophyll content and the concentration and content of a range of structural and nutritional elements.

(2) Depending on the host age at inoculation, the effect of the nematode on APS rate showed as early as 3 d after inoculation and chlorophyll and the elements one week after nematode inoculation.

(3) In most cases, the effect of the nematode increased with level and duration of infection, particularly the effect on APS rate. Moreover, the younger the plants were at the time of nematode inoculation the more they suffered from nematode infection.

(4) Identifies the relationship between the changes in the concentrations and distribution of the elements and changes in
host physiology, which suggests a role of elements in the overall physiological processes and host productivity.

(5) Identifies potassium as one of the 'root-derived factors' affecting photosynthesis that is modified by nematode infection. This is a significant step towards our understanding of the mechanisms by which nematodes affect the photosynthetic process.

(6) Establishes the relationship between the above physiological processes and their effect on host morphology as influenced by the nematode and, that this interaction of the physiological processes and morphological characteristics that results in yield loss.

(7) Provides useful quantitative information that could be used for crop protection strategies and development of predictive models for nematode control. Furthermore, the results provide a basis upon which an improved potassium fertilizer programme could be developed in order to compensate for nematode induced loss of yield.

(8) The results suggest an interaction between the nutrient elements and plant growth regulators, such as cytokinins and gibberellins, that alters the host metabolism and translocation processes thereby diminishing host productivity.


Journal of Nematology 11, 156-161.


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Stump, I. G., Kearney, J., D'Auria, J. M. & Popham, J. D.


in the internal limitations to bean-leaf photosynthesis: CO2 dependence of net photosynthetic rate. *Photosynthetic*a 14, 489 - 496.


Appendix I - A summary description of the basic components of the computer system and software used for data collection and analysis of the CO₂ exchange rate measurements
The system runs on a PDP-8e (INTERSIL 6100) with 24k memory and two, single-density, floppy disc drives. The resources available that require control software are: (1) The ES-130 48 channel 12 bit A-D and (2) the CONTROLLED OUTLET box with eight 110 VAC 1 AMP outlets. These devices allow the system to control up to eight low power AC devices and acquire data from a wide variety of devices that produce a voltage output proportional to some time-varying analog value. It was necessary that a system be devised that would integrate control of these devices, was simple to use and was sufficiently flexible to cover a wide number of possible applications. Hence, it was decided to use the DATA-ACQUISITION SOFTWARE system and modify it to give the required features.

The purpose of this software is to provide a basic operating system for gathering data from the ES-130, controlling the devices plugged into the CONTROLLED OUTLET BOX, storing the data gathered on disc and retrieving the data again for display or analysis at a later time. The software consists of (1) DATA ACQUISITION and CONTROL and (2) DATA ANALYSIS programs.

The DATA ACQUISITION and CONTROL part comprises the ADSET.SV, ADGET.SV, ADRUN.SV, ADPRT.SV and CURVS.SV programs that are stored in the SYSTEM DISC. The ADSET program is used to create the DATA-CONTROL FILES, which are general purpose files which may be regarded as a TEMPLATE used by the rest of the system for control purposes, and allows the user to specify the following: (1) The number of A-D channels to digitize. (2) The
order in which the A-D channels will be scanned. (3) A label consisting of up to six alpha-numeric characters that identifies data from each channel. A second label to identify the type of units the data is to be reported in. i.e.: DEG C, PPM, % CO2, etc. (4) The number of EVENT SEQUENCES to be done. (5) The time duration each controlled device will be activated. (6) The overlap time between consecutive event sequences. (7) The delay from the time the device is activated until the first A-D scan. (8) The time delay between successive A-D scans.

This information is stored in a DATA-CONTROL FILE in the format shown below.

<table>
<thead>
<tr>
<th>Chamber #</th>
<th>Run time (s)</th>
<th>Overlap time (s)</th>
<th>Time when data collection starts (s)</th>
<th>Time interval between scans (s)</th>
<th>Number of scans</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>-10</td>
<td>90</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>-10</td>
<td>90</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>-10</td>
<td>90</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>-10</td>
<td>90</td>
<td>30</td>
<td>7</td>
</tr>
</tbody>
</table>

The user can recall the DATA-CONTROL FILE using the ADGET program which includes the CURVS program that allows the user to create LINEARIZATION DATA FILES for the temperature and CO2 calibration curves. These files contain the data used by the CALIBR section of ADGET when the coefficients for the least squares fit to a polynomial is done in order to linearize non linear devices. The data are organized in pairs of numbers where
each pair represents a point on the curve of the device in question. The user is required to supply the name for the LINEARIZATION. It should be noted here that there is a list of devices in ADGET, and that it will be easier for the user to remember the LINEARIZATION DATA FILE name if he uses the same names as are in the list in ADGET. The system allows for up to 30 pairs of numbers to describe the transfer characteristic of any non-linear device. The user must specify how many number pairs there will be in the file he is creating.

The user can view what is in the file by recalling the DATA-CONTROL FILE and printing. Necessary changes can be made if the following format is not to the satisfaction of the user.
Valve / A-D DATA-CONTROL summary:

<table>
<thead>
<tr>
<th>List and # of Assigned Values</th>
<th>Calibration</th>
<th>for measured curves</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-D channels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-D channel #1</td>
<td>Temp1(^1)</td>
<td>Deg C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type-T(^2)</td>
</tr>
<tr>
<td>A-D channel #2</td>
<td>Temp2</td>
<td>Deg C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type-T</td>
</tr>
<tr>
<td>A-D channel #3</td>
<td>Temp3</td>
<td>Deg C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type-T</td>
</tr>
<tr>
<td>A-D channel #4</td>
<td>Temp4</td>
<td>Deg C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type-T</td>
</tr>
<tr>
<td>A-D channel #5</td>
<td>Temp5</td>
<td>Deg C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type-T</td>
</tr>
<tr>
<td>A-D channel #6</td>
<td>Temp6</td>
<td>Deg C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type-T</td>
</tr>
<tr>
<td>A-D channel #7</td>
<td>CO(_2)</td>
<td>PPM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Linear</td>
</tr>
</tbody>
</table>

Valve Valve Overlap A-D A-D Inter- # A-D
sequence time time (s) delay scan delay scan (s) (s)

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>300</td>
<td>-10</td>
<td>90</td>
<td>30</td>
<td>7</td>
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<tr>
<td>2</td>
<td>300</td>
<td>-10</td>
<td>90</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>-10</td>
<td>90</td>
<td>30</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>-10</td>
<td>90</td>
<td>30</td>
<td>7</td>
</tr>
</tbody>
</table>

Note: 1 Temp1 - 5 refer to the temperature control in each chamber and Temp6 to room temperature.

2 Type-T refers to the calibration curves for temperature.
After making the necessary changes, entering the time the schedule to begin and the description of the experiment, the user can execute the RUN (which is part of the ADRUN program). All the above information is written into a DATA-INFO FILE and the user is prompted for a DATA-SERIES name of the following form:
CCCCNN where, C = any alphanumeric character and N = any number 0-9. A more usual response would be to supply a DATA-SERIES NAME such as TREE00 to create the DATA-SERIES:
TREE00.DA ........ DATA-INFO FILE
TREE01.DA ........ DATA-SERIES FILE
TREE02.DA ....... etc. Note: The '.DA' extension is automatically supplied by the system.

The DATA-INFO file is included to aid in documentation of any experiment where the data acquisition system is used. The DATA-INFO file (the first file in the DATA-SERIES) will contain: (1) all of the information from the DATA-CONTROL file used, (2) the time to start the schedule, (3) the DATE the system was started, (4) a 5 lines, up to 78 characters per line, description of the experiment (optional) and (5) the calibration data for the A-D. The sequentially numbered files that follow all contain data from the A-D along with time information. If the operator desires to suspend A-D activity without losing data, he/she may enter CNTRL H. The system will send the buffer to the disc and end the FILE-SERIES.
Once an experiment has been completed, there will be a series of files on the data disc which contain the RAW DATA for CO₂ and TEMPERATURE. The formula for photosynthetic rate measurement (see Sestak et al., 1971) was plugged into the system and there are three programs required to do the data analysis and calculations; viz. ADEDT.SV, ADSEP.SV and ADANA.SV.

The ADEDT PROGRAM provides the user with the ability to:

(1) Enter the "TAG NUMBERS" of records to be deleted.

(2) Enter the "TAG NUMBERS" where a flow value is to be inserted and the value of flow to be inserted.

(3) Barometric pressure in a manner similar for flow rate.

(4) Sort the table thus entered so that the "TAG NUMBERS" are in order.

(5) Print the table.

(6) Save the table.

(7) Recall a table previously created and

(8) Delete any record in the table. The ADEDT modification was done to give the user the capability of making a deletion of data associated with a whole range of TAG NUMBERS. This was done by setting up a new type of action pointer. The information specifying the range of TAG NUMBERS was entered in the FLOW and PRESSURE values. The printed table was modified and the SORT OPERATION was expected to find errors that could arise due to the new type of errors pointed. The capability to send the table to the LPT: was included as well as the time.

The ADSEP PROGRAM is run to create files containing averaged values for the DATA from each chamber from which data were collected. The ADEDT PROGRAM must have been used previously.
used to create a file containing EDIT-DATA for the data to be processed as ADSEP. The EDIT-DATA FILE should contain the TAG NUMBERS of all RAW DATA RECORDS to be deleted as well as the TAG NUMBERS where FLOW and PRESSURE DATA values are to be inserted into the records produced by ADSEP. The ADSEP PROGRAM puts the data it produces into a series of FILES that have the FORM XXXX00.DA, XXXX01.DA, XXXX02.DA etc. These names are supplied by the PROGRAM and not by the user. They can, however, be re-named afterwards if they are to be saved permanently. The DATA FILES produced by ADSEP can then be processed by ADANA to get final results.

The ADANA program processes data in the format produced by the ADSEP program. The program will read up to 200 records. If there are more than 200 records, only 200 records will be read and processed. Each data record is read from the file into a DATA BUFFER in memory. The data record format is: CHAMBER #, TIME, TEMPERATURE, CO2, FLOW, PRESSURE. The data records can be processed and the results from each chamber can be separated from the data for the other chambers. Photosynthetic rate can be calculated for each record in each chamber. The user has the choice to base the calculation on any plant parameter he desires. i.e. leaf area, leaf chlorophyll, dry weight etc. Mean and standard deviation for each chamber are calculated and reported.
APPENDIX II - Leaf sample storage and chlorophyll extraction and analysis
(1) Sample collection and storage

For chlorophyll analysis, a known number (5 - 10) of leaf discs were collected, using a 5.5 mm diameter cork borer, from alternate sides of sampled leaves. To prevent photo-oxidation (Linder, 1974), each leaf disc was placed flat with fine forceps on black plastic between two, labelled microscope slides. Each sample was wrapped separately with a rubber band (Viceroy Manufacturing Co. Ltd., Canada), and kept frozen in dark boxes until extraction.

(2) Extraction

Extractions were done in 100 % acetone containing a drop of MgCO₃ to buffer the solution (Linder, 1974). The dark boxes containing the leaf discs, Erlenmyer filtering flasks (Fisher Scientific) (one for each treatment), graduated cylinders, a grinding vessel (Arthur, H. Thomas Co., Philadelphia, USA), prelabelled 30 ml vials and the acetone in squirt bottles were placed on ice so that degradation of chlorophyll was minimized (Linder, 1974) (see Fig. 37).

Leaf discs from each of the boxed samples were removed with forceps under safe (green) light, and placed individually, into the grinding vessel. Approximately, 1 - 2 ml of acetone and a pinch of burned acid-washed fine sand were added to the grinding tube. Each sample was ground for one minute at 1550 rpm using a motorized tissue grinder, model 5VA (Eastern Industries, Hamden, Conn.) fitted with Teflon Resin Pestle (Potter Elvehjem Tissue
Grinder, Fischer Scientific). The suspension was washed out of the grinding vessel with the solvent and poured into a Buchner Type Porcelain Funnel fitted with Whatman # 1 (5.5 cm diameter) filter paper and filtered for one minute into their respective Erlenmyer flasks by vacuum suction. The filtered extracts were poured into graduated cylinders, volumes standardized (20 - 25 ml) and stored in vials in an ice box. The grinding vessel, pestle, Erlenmyer flasks, graduated cylinders and Buchner funnel were thoroughly rinsed with acetone between each processing.

(3) Measurement and calculations

Beckman model 35 (USA Ltd.) spectrophotometer was used for chlorophyll a & b determinations and, prior to taking readings, it was zeroed by putting 3 ml of 100 % acetone into the reference and sample cuvettes separately. A 3 ml extract was placed into the sample cuvette and chlorophyll a & b measurements taken at 662 and 644 nm respectively, over a continuous spectral region from 700 - 400 nm (Linder, 1974). The time elapsed between extraction and the spectrophotometric reading of each sample was approximately 2 hr during which time the samples were kept in ice. Data was calculated using the formulae of Arnon (1949) and Holm (1954).
Figure 37
Diagramatic representation of the procedures used during chlorophyll extraction and analysis.
Appendix III - Publications arising from the thesis
The following papers have been published based on research described in the thesis.


Paper No. 1 is based mainly on the techniques as described in chapter II, the General Materials and Methods, and in Appendix I. Paper No. 2 is based on chapter III. Papers No. 3 and 4 (both in press) are based on chapters IV and V, respectively.