

1.02
1000 E/P
KTN

**INTERRELATION BETWEEN ROOT-KNOT
NEMATODE MELOIDOGYNE INCOGNITA
AND VESICULAR-ARBUSCULAR
MYCORRHIZA ON EGYPTIAN CLOVER**

BY

KHALED MOHAMMED ABDEL KADER

B.Sc. (Agriculture, Entomology)

THESIS

SUBMITTED IN PARTIAL FULFILMENT

OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

IN

AGRICULTURAL ZOOLOGY

**Plant Protection Department
FACULTY OF AGRICULTURE
AIN SHAMS UNIVERSITY**

1987

BIOGRAPHY

Name : Khaled Mohammed Abdel Kader.
Degree : B.Sc. Agriculture-Entomology.
Occupation : Demonstrator in Plant Protection Department,
Faculty of Agriculture, Ain Shams University.
Date of Appointment : 17/10/1983
Date of Registration: 16/1/1984

| Courses Attended : | Units | Grade |
|-----------------------------------|-------|-----------|
| Nematology A(201) | | |
| Morphology & Taxonomy | 4 | Excellent |
| Nematology B(202) | | |
| Economic Nematology | 3 | Excellent |
| Soil Fauna (206) | 3 | Excellent |
| Nematode Diseases of Plants (212) | 4 | Excellent |
| Soil Microbiology (210) | 4 | Very Good |
| Taxonomy of Fungi (207) | 4 | Good |
| Plant Microtechnique (203) | 4 | Good |
| Special Studies (298) | 3 | Excellent |
| Research (300) | 5 | Excellent |
| English Language | - | Pass |



ACKNOWLEDGMENT

The author wishes to express his gratitude and sincere appreciation to Dr. A. H. Y. Taha, Professor of Agricultural Zoology, Department of Plant Protection, Faculty of Agriculture, Ain Shams University, for suggesting the topic of this study, for his guidance and supervision in the course of the work, and for his stimulating criticisms and help in the preparation of the manuscript.

The author is also deeply indebted to Dr. E. M. Ramadan, Professor of Agricultural Microbiology, at the same Faculty, for his suggestions during the initial phase of the study.

The author is grateful to Dr. A. S. Kassab, Lecturer in Agricultural Zoology, Department of Plant Protection, same Faculty, for his keen interest and suggestions for histological studies.

Special thanks are due to Mr. Kh. El-DougDoug, Assistant Lecturer, Department of Microbiology, for his help in statistical analysis, Mr. M. Abd-El-Fattah, Assistant Lecturer, Department of Soils, and Mr. H. El-Shouray, Assistant Lecturer, Department of Horticulture, for their help in chemical determination.

TABLE OF CONTENTS

| | Page |
|--|------|
| I. INTRODUCTION | 1 |
| II. REVIEW OF LITERATURE | 3 |
| A. The Potential Significance of Mycorrhizal Association in Crop Production..... | 3 |
| B. The Importance of Vesicular-Arbuscular Mycorrhizae to plants | 4 |
| C. Effect of Vesicular-Arbuscular Mycorrhizae (VAM) on Rhizobia | 7 |
| D. The Relationship Between The Host Plant And Root-Knot Nematode And Associated Physiological Changes | 10 |
| E. Root-Knot Nematodes And Nodulation | 13 |
| F. Interaction Between Vesicular-Arbuscular Mycorrhizae (VAM) And Root-Knot Nematodes | 15 |
| III. MATERIALS AND METHODS | 18 |
| A. Soil Texture | 18 |
| B. Plant Host | 18 |
| C. Rhizobium Inoculum | 18 |
| D. Nematode Source And Inoculum | 19 |
| E. Source of Endotrophic Vesicular-Arbuscular Mycorrhizae (VAM) | 19 |

| | |
|---|----|
| F. Experiments | 20 |
| 1. Interaction between vesicular-arbuscular mycorrhizae (VAM) and <u>Meloidogyne incognita</u> on nodulated clover plants | 20 |
| 2. Determination of the possibility of VAM and <u>Meloidogyne incognita</u> to invade clover nodules | 21 |
| G. Collection of Data | 22 |
| 1. Plant growth | 22 |
| 2. Scoring of mycorrhizal and nematode infection | 22 |
| a. Nematode infection | 22 |
| b. Mycorrhizal infection | 22 |
| H. Histological Procedures | 22 |
| K. Chemical Determination | 23 |
| 1. Total nitrogen in shoots | 23 |
| 2. Phosphorus content of shoots | 23 |
| L. Statistical Treatment of Data | 23 |
| IV. RESULTS | 24 |
| A. THE EFFECT OF VESICULAR-ARBUSCULAR MYCORRHIZAE (VAM) ON MELOIDOGYNE INCOGNITA INFECTION ON CLOVER PLANTS | 24 |
| 1. Plant Growth | 24 |
| 2. Total Nitrogen | 24 |

| | |
|---|----|
| 3. Phosphorus Content | 27 |
| 4. <u>Meloidogyne incognita</u> Galls and Population.. | 27 |
| a. Galls | 27 |
| b. Population | 29 |
| 5. Mycorrhizal Root Length (%) | 29 |
| B. THE EFFECT OF THE PREINOCULATION WITH EITHER VESICULAR-ARBUSCULAR MYCORRHIZAE (VAM) OR MELOIDOGYNE INCOGNITA ON THE POSTINOCULATION WITH THE OTHER ORGANISM | 30 |
| 1. Preinoculation With Vesicular-Arbuscular Mycorrhizae (VAM) | 30 |
| a. Plant Growth | 30 |
| b. Total Nitrogen | 30 |
| c. Phosphorus Content | 30 |
| d. <u>Meloidogyne incognita</u> Galls and Population | 30 |
| i. Galls | 30 |
| ii. Population | 34 |
| e. Mycorrhizal Root Length (%) | 34 |
| 2. Preinoculation With <u>Meloidogyne incognita</u> .. | 35 |
| a. Plant Growth | 35 |
| b. Total Nitrogen | 35 |
| c. Phosphorus Content | 35 |
| d. <u>Meloidogyne incognita</u> Galls and Population | 39 |

| | |
|--|----|
| i. Galls | 39 |
| ii. Population | 39 |
| e. Mycorrhizal Root Length (%) | 39 |
| C. WHOLE MOUNT AND HISTOLOGY OF CLOVER ROOTS AND NODULES INFECTED WITH VESICULAR-ARBUSCULAR MYCORRHIZAE (VAM) AND MELOIDOGYNE INCOGNITA .. | 41 |
| V. DISCUSSION | 42 |
| VI. GENERAL CONCLUSIONS | 48 |
| VII. SUMMARY | 50 |
| VIII. LITERATURE CITED | 52 |
| ARABIC SUMMARY. | |

I. INTRODUCTION

The root system of higher plants is associated not only with an inanimate environment composed of organic and inorganic substances but also with a vast population of metabolically active microorganisms. The plant creates a unique subterranean habitat for microorganisms; and, in turn, is markedly affected by the population it has stimulated since the root zone is the site from which mineral nutrients are obtained and through which pathogens must penetrate. Consequently, interaction between the plants and the microorganisms in this locale can have a considerable significance for crop production and soil fertility.

Several relationships between the plants and their rhizosphere inhabitants can be recognized. Symbiotic phenomena are observed between legumes and rhizobia and in the mycorrhizal associations. Pathogenic relationships are not uncommon because roots are attacked by a number of fungi, bacteria, and nematodes. Rhizobial and mycorrhizal associations contrast with parasitic infections by the prolonged period of healthy physiological interaction between rhizobia or mycorrhizae and host plant.

The contribution of legumes to the maintenance of soil fertility has been of peculiar interest to agricultural scientists. The ability of leguminous plants to obtain their nitrogen from the atmosphere is dependent on the presence

11

of rhizobia of a specific group which under suitable conditions stimulate the plants to form rhizobial nodules on the roots within which the rhizobia grow and multiply.

Mycorrhizae, especially endomycorrhizae of the vesicular-arbuscular (VAM) type, occur in virtually every plant family. Most of the important crop plants are capable of forming such associations. Their benefit to plants is easily demonstrated under poor nutritional conditions.

The most obvious effect of nematode parasitism is growth reduction. The diversity of symptoms incited suggests a variety of mechanisms, all involving impairment of root function in the case of root nematodes. The root-knot nematode, Meloidogyne incognita (Kofoid & White) Chitwood, for example, disrupts root function and nutrient absorption, reduces number of feeder roots, and suppresses plant growth and yield (Hussey, 1985).

The objective of the present study was to determine the reciprocal effect of M. incognita and vesicular-arbuscular mycorrhizal fungi (mainly Glomus spp.) infections on:

- 1) the growth of Egyptian clover (Trifolium alexandrinum L.) plants;
- 2) number of nematode stages and final population;
- 3) the rate of mycorrhizal infection (%);
- 4) total-N and P-content of clover shoots; and
- 5) the histology of the nodule and nematode gall.

II. REVIEW OF LITERATURE

A. The Potential Significance of Mycorrhizal Associations in Crop Production

As summarized in a review article by Ruehle and Marx (1979), the general majority of plants, including most economically important crops, form mycorrhizal associations in which the mycobiont takes over, either partially or completely, the various functions of root hairs. Much more widespread are endomycorrhizae, in which the mycobiont enters the cortical cells of the host plant producing fungal structures, viz., vesicles and arbuscules, but usually do not affect the gross morphology of the root (Bartha, 1980). Externally to the root, a loose web of mycelium occurs. This may be differentiated into long and short branches and may bear vesicles, spores, or fruit bodies. The hyphae are aseptate except where vesicles or fine lateral branches are cut off (Harley, 1965). These are mostly of the vesicular-arbuscular endomycorrhizae (VAM). The mycobionts of VAM are most commonly members of the family Endogonaceae. The most important genera in this family are Glomus, Gigaspora, Acaulospora and Sclerocystis. They appear to have wide distribution, both geographically and in terms of soil types.

B. The Importance of Vesicular-Arbuscular Mycorrhizae to Plants

Numerous laboratory and several field experiments have demonstrated that VAM association can greatly improve plant growth and nutrition. Safir and Nelsen (1980) stated that mycorrhizal root systems are generally more efficient at taking up nutrients. Under nutritionally restrictive conditions, mycorrhizal plants have greater access to inorganic nutrients, especially to those that have limited solubility, and mobility, e.g., phosphate, zinc, molybdenum, and ammonia (Bartha, 1980). The mycobiont receives photosynthate from the plant. The probable source of energy for hyphal uptake of nutrients is most likely obtained from the host in the form of organic compounds (Safir & Nelsen, 1980).

Four hypotheses have been proposed by Safir and Nelsen (1980) to explain the improved nutrition of mycorrhizal plants. These hypotheses were discussed primarily in relation to phosphorus nutrition, because the increased uptake of phosphorus is usually associated with the improved growth of mycorrhizal plants.

The first hypothesis is that the mycorrhizal root surface is a more efficient nutrient absorber, that is, physiological changes due to infection occur in the

infected root causing it to more readily absorb soil nutrients. Gray and Gerdemann's study (1969) supported this hypothesis using radioactive phosphorus. They found that noninfected segments of inoculated roots absorbed more phosphorus than noninoculated check roots. However, the infected segments of mycorrhizal roots absorbed much more phosphorus than did uninfected segments.

The second hypothesis is that mycorrhizal root segments are able to use nutrients sources that are unavailable or less available to nonmycorrhizal root. Murdoch et al. (1967) demonstrated that mycorrhizal corn plants were larger and had higher phosphorus contents than did nonmycorrhizal corn when phosphorus sources of low availability (rock phosphate) were added to soil. Conversely, mycorrhizal and nonmycorrhizal plants had equal phosphorus contents and grew equally well with a readily available phosphorus supply. Similar results have been obtained by Ross and Gilliam (1973) and Powell and Daniel (1978). However, studies by Sanders and Tinker (1971), Hayman and Mosse (1972) and Powell (1975) indicated that mycorrhizal and nonmycorrhizal plants were using the same sources of phosphorus. It appears that the increased utilization by mycorrhizal plants of low-availability phosphorus sources can largely be explained by

an increased uptake rate by mycorrhizae of phosphorus in soil solution (Safir & Nelsen, 1980). Fares (1986) found that mycorrhizal peanut plants not fertilized with phosphate had approximately the same morphological characteristics and pod yield as those of fertilized nonmycorrhizal plants.

The third hypothesis is that the soil network of mycorrhizal hyphae is able to absorb nutrients from larger soil volume and translocate them to the infected roots. Hattingh et al. (1973) demonstrated that mycorrhizal hyphae are capable of transporting large amounts of phosphorus to mycorrhizal roots. This was confirmed by Rhodes and Gerdemann (1978) who used a split plate technique to separate mycorrhizal plant roots from areas of soil into which external hyphae had penetrated. Phosphorus-32 was added to the soil containing the hyphae, and ^{32}P uptake by plant roots was measured. Severing the external hyphae from the roots eliminated ^{32}P transport to the roots.

The fourth hypothesis is that mycorrhizal root segments remain functional as nutrient absorbers longer than do nonmycorrhizal segments. This hypothesis suggests that mycorrhizal infection alters root morphology to enable the entire root system to be larger and more efficient

at nutrient absorption. Safir and Nelsen (1980) stated that it is unlikely hypothesis because root-shoot ratios are generally lower for mycorrhizal plants than for non-mycorrhizal plants. It is also unlikely that mycorrhizal roots take up large amounts of phosphorus because they remain active longer than nonmycorrhizal roots, for mycorrhizal growth stimulation may occur several weeks after inoculation.

C. Effect of Vesicular-Arbuscular Mycorrhizae (VAM) on *Rhizobia*

VAM infection has been associated with improved nitrogen fixation by *Rhizobium*. Greenhouse experiments conducted by Daft and El Giahmi on bean (1974) and peanut (1976) showed that mycorrhizal fungi in plant roots increased plant growth, seed yields, number of nodules, and acetylene reduction rates over plant that had no mycorrhizal fungus present. They further demonstrated that most but not all this increase was due to the increase in phosphorus levels in mycorrhizal plants. Bagyaraj et al. (1979) demonstrated that *Glomus fasciculatus* could significantly increase the nodules on soybean on a phosphorus-deficient soil in the field, but that this increase did not result in a significant increase in seed yield. Fares (1986) found that all peanut plants