M.A. RUISSEN

THE DEVELOPMENT AND SIGNIFICANCE OF VESICULAR-ARBUSCULAR MYCORRHIZAS AS INFLUENCED BY AGRICULTURAL PRACTICES

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee,
hoogleraar in de veeteeltwetenschap,
in het openbaar te verdedigen
op vrijdag 19 november 1982
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

ISN: 178283-03

THE DEVELOPMENT AND SIGNIFICANCE OF VESICULAR-ARBUSCULAR MYCORRHIZAS AS INFLUENCED BY AGRICULTURAL PRACTICES

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Co-referent: dr.ir. T. Limonard, wetenschappelijk hoofdmedewerker

STELLINGEN

I

Voor een beter begrip van het functioneren van mycorrhiza's dient men zich rekenschap te geven dat symbiose geen synoniem is van mutualisme.

II

Bij bodemvruchtbaarheidsonderzoek zowel als in veredelingsprogramma's die gericht zijn op produktieverbetering in landbouwkundig gezien marginale gebieden dient men terdege rekening te houden met de potentiële mogelijkheden die vesiculair-arbusculaire mycorrhiza's bieden.

III

Bij eventuele nieuwe inpolderingen dient enten van de grond met mycorrhizaschimmels overwogen te worden.

IV

Bemesting met minder goed oplosbare, natuurlijke fosfaten biedt, eventueel in combinatie met symbiontische stikstofbinders en mycorrhiza's, duidelijke maar beperkte mogelijkheden ter vervanging van het relatief dure superfosfaat.

V

De totaal verschillende organismen die in de afgelopen jaren als vermoedelijke oorzaak van de groeidepressies in nauwe rotaties zijn aangemerkt, illustreren het gebrekkige inzicht in de ecologie van bodemmicro-organismen.

VI

Het onverwacht sterk optreden van bacterievuur in Zuidwest-Nederland in de zomer van 1982 wijst op een onderschatting van de dreiging die uitgaat van bacterieziekten in land- en tuinbouw.

VII

Biologische bestrijding van "bodempathogenen" als een alternatief voor bestaande en verantwoorde chemische bestrijding, heeft alleen kans van slagen indien optimaal aan de levensvoorwaarden van de toegepaste organismen kan worden voldaan.

VIII

Ten behoeve van de studie van bodemecosystemen is het wenselijk om analoog aan de plantenecologie te komen tot een classificering van landbouwgronden op basis van kenmerkende, in de grond aanwezige micro-organismen.

IX

De toepassing van boomchirurgie is maar al te vaak dure kwakzalverij.

X

Het verdient aanbeveling de uitvoering van de door Nederland beoogde ontwikkelingssamenwerking te laten berusten bij een politiek zo onafhankelijk mogelijke instelling.

XI

De ontwikkeling van landbouwmethoden waarbij men streeft naar integratie van verworvenheden uit zowel gangbare als alternatieve vormen van landbouw is wenselijk vanuit het oogpunt van het veilig stellen van onze Nederlandse landbouwbelangen op langere termijn.

Proefschrift van M.A. Ruissen.
19 november 1982.

CONTENTS

WOORD VOORAF

Met het gereedkomen van dit proefschrift wil ik graag diegenen bedanken die bij de totstandkoming ervan hebben bijgedragen. Daarbij gaat de dank in de eerste plaats naar mijn ouders die mij vooral in de eerste fase van mijn opleiding hebben gestimuleerd waardoor een voortgezette opleiding in Wageningen mogelijk werd. Voor Uw steun en belangstelling die ik hierbij heb ondervonden ben ik U zeer erkentelijk.

Mijn dank gaat ook uit naar prof.dr.ir. J. Dekker, mijn promotor, die mij de mogelijkheid heeft geboden om bij de vakgroep Fytopathologie dit proefschrift te bewerken. De vrijheid die ik kreeg gedurende het onderzoek, Uw belangstelling voor het verloop van het onderzoek en de kritische begeleiding bij de afronding ervan heb ik zeer gewaardeerd.

Een woord van waardering is ook bestemd voor mijn co-referent, dr.ir. T. Limonard. Theunis, ik vond het een genoegen om met je samen te werken en de discussies die we voerden hebben in belangrijke mate bijgedragen tot het verloop van het onderzoek. Ook wil ik je danken voor je bijdrage bij het op schrift stellen van dit proefschrift.

De Stichting Proefstation voor de akkerbouw en de groenteteelt in de volle grond (PAGV) en het Instituut voor Bodemvruchtbaarheid (IB) dank ik voor de gelegenheid die geboden werd op hun proefbedrijven respectievelijk te Nagele en Marknesse materiaal te verzamelen voor dit onderzoek.

Pieter Vereijken dank ik voor het motiverende, enthousiaste meedenken gedurende het onderzoek en de aanvullende informatie betreffende de bedrijven in Nagele. Met jouw dank ik ook de heer J. van de Westeringh die als bedrijfsleider van deze bedrijven telkens met raad en daad behulpzaam was.

De heren ir. J.A. Grootenhuis en J.K. Mulder dank ik voor de medewerking die ik ondervond op de Lovink-hoeve.

Gedurende het onderzoek waren een aanzienlijk aantal studenten betrokken bij het mycorrhiza onderzoek. Een deel van

hen heb ik met veel genoegen mogen begeleiden bij hun doctoraalonderwerp. Met name wil ik hier noemen Tarsy Lössbroek, Liesbeth Goense-Wagemaker, Ron Poppelaars, Menno van Hulst, Anne-Maria Wagner (IAESTE-stagiaire) en Gerda Peters (Stova-stagiaire) die een bijdrage leverden voor dit proefschrift.

De gastvrijheid en de technische faciliteiten die telkenmale geboden werden door de vakgroep Nematologie heb ik zeer op prijs gesteld.

De firma Zelder B.V. te Ottersum stelde telkens weer belangeloos zaaizaad voor kasproeven ter beschikking.

De afdeling tekstverwerking, en met name de dames H. D'hondt-de Jong en W.M. Laoh-Gieskes, en de offsetdrukkerij van de Landbouwhogeschool droegen zorg voor het typewerk en de vermeerdering ervan. F.J.J. von Planta ontwierp de omslag van het geheel.

Liesbeth, jou wil ik in dit voorwoord zeker niet vergeten. Niet alleen omdat jij het verloop van het onderzoek en het schrijven van het proefschrift van heel nabij hebt meegemaakt, maar ook omdat je, op veel verschillende wijzen, een bijdrage leverde bij de totstandkoming ervan.

Tenslotte wil ik allen die niet in dit voorwoord vermeld zijn, maar toch belangstelling toonden voor mijn onderzoek bij deze hartelijk danken.

LIST OF ABBREVIATIONS

farms

DOB-farms		Drie Organische stof Bedrijven = = Three Organic Matter Farms
	KA	Kunstmestakker = Fertilizer field
	WW	Wisselweide = Rotational pasture
	KL	Klaverland = Clover field
OBS-farms		Ontwikkeling Bedrijfssystemen = = Development of Farming Systems
	GA	Gangbaar bedrijf = Current farm
	GI	Geïntegreerd bedrijf = Integrated farm
	BD	Biologisch-dynamisch bedrijf = Bio-dynamic farm

crops

P	potato
WW	winter wheat
SW	spring wheat
SB	sugarbeet
FB	fodderbeet
B	spring barley
O	oats
ON	onion
G	pasture
F	flax
L	ley

miscellaneous

VAM	vesicular-arbuscular mycorrhiza
FYM	farmyard manure
p	probability
s.d.	standard deviation

1 *INTRODUCTION*

Soil micro-organisms are of great importance in agriculture, both as a threat to the health of plants and as an ally in efforts to improve crop production. Many micro-organisms live in close association with crop plants. De Bary (1879) defined the living together of unequal organisms as symbiosis. This symbiosis may be disadvantageous to the host plant (parasitism) or of advantage to it (mutualism).

One of the symbiotic relations between micro-organisms and plants is mycorrhiza, that is generally a mutualistic association between a fungus and plant roots. Two main types of mycorrhizas are distinguished, ectomycorrhizas and endomycorrhizas. The ectomycorrhizas consist of a thick sheath of dense mycelium around the feeder roots of plants, mainly of trees. The mycorrhizal fungus involved is usually unable to invade the cells of the host. In the case of endomycorrhizas, the fungus forms only a loose mycelium around the roots and invades the cells of the root cortex. A special form of endomycorrhizas is the vesicular-arbuscular mycorrhiza (VAM), which occurs in many important agricultural crop plants. In the last twenty years there have been numerous reports of the positive effect of these mycorrhizas on the growth of plants. Their role in agriculture is, however, little understood and needs further elucidation.

The purposes of this study are to assess the occurrence of VAM in agricultural crops, particularly in wheat and potatoes; to obtain information about the effect of farming systems and agricultural practices on the development of VAM, and to gain an insight into the role which VAM may play in agricultural crops. Finally the question is considered as to whether measures should be advocated to stimulate the development of VAM in agricultural crops.

Three neighbouring experimental farms (DOB/OBS-farms) in the vicinity of Nagele in the North East Polder, The Netherlands, provided an opportunity to study the occurrence of VAM in farming systems in which during a period of 26 years different applications of organic matter have been made. In addition it provided an opportunity to compare the development of VAM in the different farming systems in a new design of these farms established in 1979. The development of VAM in the new situation was of particular interest because on two of the farms no or only limited amounts of fertilizers and plant protection chemicals were used.

An outline of VAM is given in chapter 2 and the experimental farms are described in chapter 3. The observations made on the DOB/OBS-farms are presented in chapter 4. In order to obtain a better understanding of the differences observed between the farming sytems, additional information on the effect of various agricultural practices on the development of VAM obtained from the Lovink-farm, another experimental farm in the North East Polder, is presented in chapter 5. This is supplemented in chapter 6 with a report on greenhouse experiments on the development of VAM and the significance of VAM on plant growth. The relation between VAM, fungal pathogens and disease management received attention in chapter 7.

2 *VESICULAR-ARBUSCULAR MYCORRHIZAS*

2.1 INTRODUCTION

Among the different types of mycorrhizas that are distinguished, the vesicular-arbuscular mycorrhizas (VAM) are most common (Gerdemann, 1968; Meyer, 1973). These mycorrhizas are characterized by the formation of hyphal swellings (vesicles) and by finely branched structures (arbuscules) in the root cortex cells of the hostplant (fig. 2.1). Outside the root, relatively large sized (about 50-400 µm) and thick walled chlamydospores may be found. Some VAM fungal species form clusters of chlamydospores, embedded in a matrix of hyphae (sporocarps).

Several well documented reviews, which cover various aspects of the work done on these mycorrhizas are available (Nicolson, 1967; Gerdemann, 1968; Mosse, 1973; Gerdemann, 1975; Slankis, 1974; Hayman, 1978; Smith, 1980).

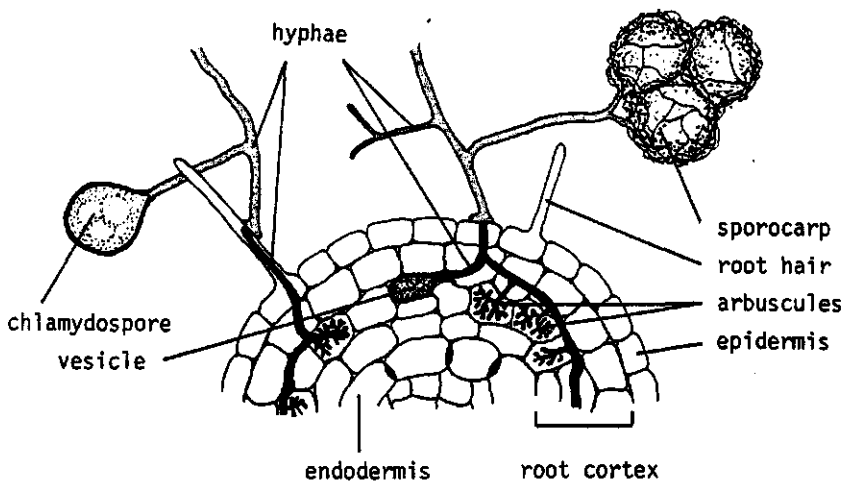

Figure 2.1 Schematic illustration of a vesicular-arbuscular mycorrhiza.

The aim of this chapter is not to add a new review to this list, but to mention some aspects which may be valuable as an introduction to VAM within the scope of this study.

2.2 HISTORY

Before Frank in 1885 gave the name of mycorrhiza to the symbiotic association between a fungus and roots of plants, quite a number of observations on this phenomenon had already been reported (Kelley, 1963). The oldest known observation on VAM was done by Treub in the former Dutch East Indies (nowadays Indonesia). The fungal symbiont was described in a Dutch publication in 1885 as a *Pythium* species in the roots of sugar cane (Treub, 1885). Investigations done by Schlicht (1889), Janse (1896) and Stahl (1900) indicated that the VAM were widespread in herbaceous plants. Our present knowledge confirms these findings. By some of the early investigators it was already recognized that the VAM development is influenced by external factors. Stahl mentioned the nutrient deficiency of the substrate as a factor stimulating mycorrhizal symbiosis. Schlicht considered the humus content of the soil an important factor. They noted also that nutrient rich substrates decreased the VAM development.

During a long period, the identity of these endophytes remained unknown until in the fifties more information was obtained. An important aspect in this respect was the finding that VAM was related with certain large sized spores found in the soil (Mosse, 1953; 1956). This initiated inoculation experiments in which also growth improvements due to increased phosphorus uptake were recorded. From that time on the VAM fungi received progressively more attention.

2.3 OCCURRENCE

The occurrence of VAM in plants is so ubiquitous that it is easier to mention those species that are found to be non-mycorrhizal than the mycorrhizal ones. They occur in a large number of agricultural plants. Gerdemann (1968) mentions a number of families that are seldom mycorrhizal. Among them

are the Cruciferae and the Chenopodiaceae. Infections with VAM fungi have incidentally been found in some species of these families (Gerdemann, 1975), but the spread of these infections was sparse and probably of little importance. The distribution of VAM is world-wide and they are only absent in a few special habitats. Such habitats include very wet places and virgin soils like coal mine spoils, lava streams and newly reclaimed polder soils.

Only a few reports on the occurrence of VAM in The Netherlands are known. Henriette Bouwens (1937), who studied the mycorrhizas of fruit trees, mainly of *Cydonia vulgaris*, and strawberries, found VAM in those plants. She performed inoculation experiments which were only successful with root fragments as inoculum. In his key for the hypogeous fungi of The Netherlands, De Vries (1971) mentioned three *Endogone* species which were recovered from soils in woodlands. Only *E. lactiflua* belongs still to the genus *Endogone*, the other two species have been transferred to the genus *Glomus*. The most recent report concerns the abundance of VAM in *Plantago lanceolata* and *P. coronopus* in natural dune grassland (Van Dijk, 1980).

2.4 MORPHOLOGY AND TAXONOMY

Nowadays there are no more difficulties with the recognition of the structures of the VAM fungi in and outside the roots. The recovery of the spores (singly born or clustered in sporocarps) from the soil is often carried out by the wet-sieving and decanting method of Gerdemann & Nicolson (1963). Other methods have been described too, such as those based on sucrose-centrifugation, adhesion-flotation and the sedimentation on gelatin columns. These methods are compared by Smith & Skipper (1979).

Various staining techniques are available for the visualization of VAM fungi in the roots (Gerdemann, 1955; Bevege, 1968; Phillips & Hayman, 1970; Trappe et al., 1973). The most current one is the method of Phillips & Hayman (1970) which is based on staining the fungi with trypanblue after simmering the roots in a KOH solution. This method has been applied

during this study (4.2.3). General morphological aspects of VAM are reviewed in detail by Nicolson (1967), Gerdemann (1968, 1975), Mosse (1963) and Hayman (1978).

The taxonomy of the Endogonaceae, order Mucorales, which include the VAM fungi, is a difficult and confusing matter. This is mainly due to the scarcity of the collected material and the absence of a generative phase. The described species are form species and therefore every small difference can be a reason to describe a new species. The obligately parasitic character is another disadvantage.

An important contribution to the taxonomy of the VAM fungi is the monograph of Gerdemann & Trappe (1974), describing the Endogonaceae in the Pacific Northwest of the USA. In their booklet four genera that form vesicular-arbuscular mycorrhizas, viz. *Glomus*, *Gigaspora*, *Acaulospora* and *Sclerocystis* are described, with 30 species in total. In the period 1974 to 1981, 32 new species belonging to one of these four genera have been described. Keys are given by Gerdemann & Trappe (1974), Nicolson & Schenck (1979), Hall & Fish (1979) and Schenck & Smith (1982).

2.5 SPECIFICITY

The specificity of VAM fungi in regard to their host plant range, is very limited. Only Tolle (1958) found some indications of host specific relations. As reviewed by Mosse (1975), many inoculation experiments showed that VAM fungi could be transferred from one plant species to another. Nevertheless, there is increasing evidence that particular VAM fungi are sometimes preferentially associated with particular plant species (Mosse, 1975; Hayman et al., 1976; Schenck & Kinloch, 1980; Nemec et al., 1981; Ocampo et al., 1981).

In recently published papers differences between varieties of one host plant species in the effectiveness of the symbiosis with a particular VAM fungus are described such as the differences between various wheat cultivars (Bertheau et al., 1980; Azcon & Ocampo, 1981). These differences in specificity are expressed as differences in growth response.

Specificity of VAM fungi in regard to differences between isolates of one species and between different species, is often found. Their mutual difference and the order in effectivity, within a group of isolates tested, depend on soil type, pH, host plant and the form of applied phosphorus (Mosse, 1973; Graw, 1979).

2.6 VAM AND PLANT GROWTH

The interest in VAM is mainly based on the positive effect of the symbiosis on the growth of many economically important crop plants. This effect is predominantly found when plants are grown in soils with low or moderate fertility, especially when relatively immobile nutrients as phosphorus are in short supply. For the uptake of this type of plant nutrients, the extent of the root system is often the growth limiting factor. Plants with coarse root systems appeared to be more dependent on VAM than those with finely branched roots (Baylis, 1970; 1972; 1975). This indicates that VAM acts as an auxiliary root system.

Phosphorus, as a main plant nutrient, received much attention in relation to VAM. Mycorrhizal plants contain often higher concentrations of phosphorus in comparison to nonmycorrhizal plants. This can even happen when these plants are equal in size. Whether this extra phosphorus is luxury consumption or whether it is based on increased use of carbohydrates in the infected roots that results in higher phosphorus concentrations (Stribley et al., 1980) is still uncertain.

Evidence has been provided, that the extramatrical mycelium is responsible for the increased phosphorus uptake (Sanders & Tinker, 1971; 1973; Hattingh et al., 1973; Schoknecht & Hattingh, 1976). The same pool of labile phosphorus is used by mycorrhizal and nonmycorrhizal plants (Sanders & Tinker, 1971; Hayman & Mosse, 1972; Mosse et al., 1973; Powell, 1975). Protoplasmic streaming in the fungus transports the nutrients to the host (Cooper & Tinker, 1981), presumably in the form of polyphosphate. Uptake of other nutrients by VAM can occur, but relatively few reports deal with this aspect.

VAM is also associated with changes in distribution of dry matter between root and shoot. This may partly be explained by improved mineral nutrition.

Most records on improved plant growth are based on results obtained in greenhouse experiments, often with plants grown in sterilized substrates. Field experiments are few, but their number increases. More of these are needed for a good evaluation of the significance of VAM in crop production. In this study field observations were made on wheat and potatoes. In fieldgrown wheat (Khan, 1975) and potatoes (Black & Tinker, 1977), yield increases were obtained with VAM after inoculation.

A well known example of the application of VAM in crop production is the use of it in citrus nurseries in California and Florida (Menge et al., 1979). Addition of VAM fungi to substrates used in containerized crop production methods (Maronek et al., 1980) and the use of VAM in revegetation procedures (Reeves et al., 1979) seem to offer good prospects.

3 *THE EXPERIMENTAL FARMS*

3.1 INTRODUCTION

The investigations on vesicular-arbuscular mycorrhizas (VAM) under various agricultural practices have been carried out at experimental farms in the Noordoostpolder (North East Polder). This polder was reclaimed from the "IJsselmeer", the former "Zuiderzee" in the early forties of this century.
The experimental farms were:

- The DOB-farms, a combination of three neighbouring farms, located at Nagele, with a different regime of organic matter application (DOB = Drie Organische Stof Bedrijven = Three Organic Matter Farms). As will be described in 3.2 the DOB-farms are now used in the OBS-project (OBS = Ontwikkeling Bedrijfssystemen = Development of Farming Systems).
- The Dr. H.J. Lovink-Hoeve, located at Marknesse. This is an experimental farm, where the influence of various agricultural practices on soil fertility and productivity is studied.

In this chapter more detailed information is given about these farms.

3.2 THE DOB/OBS-FARMS

The DOB-farms were founded in 1951 directly after the first reclamation activities. These farms fell under the authority of the Institute for Soil Fertility (Instituut voor Bodemvruchtbaarheid) at Haren, Groningen. They consisted of three different farms of about 22 ha each, viz.:
(1) The KA-farm (Kunstmestakker = Fertilizer field).
On this farm a rotation of six years was used with the crop sequence of ware potatoes, winter wheat, flax, seed potatoes, sugarbeet and spring barley. On this farm no organic manure

or other organic material was applied except the root and stubble residues of the crops. This amounted to 2.3 tons of organic matter (dry weight) ha^{-1} $year^{-1}$. Cereal straw and sugarbeet tops were removed from the field. The soil fertility was maintained by means of mineral fertilizers only.
(2) The WW-farm (Wisselweide = Rotational pasture).
On this farm the same six crops were grown as on the KA-farm, but here an one year's ley was added every three years. This ley was sown after flax and after spring barley. Every year farmyard manure was applied to three of the eight fields at a rate of 30 tons ha^{-1}. The total input of organic matter was estimated at 4.6 tons (dry weight) ha^{-1} $year^{-1}$. Additional fertilizer was given in order to maintain a fertility level comparable to that of the KA-farm.
(3) The KL-farm (Klaverland = Clover field).
The same six crops were grown as on the KA-farm. In addition green manure crops were sown under flax, spring barley and after seed potatoes. Sugarbeet tops were ploughed under. The total input of organic matter was 5.7 tons (dry weight) ha^{-1} $year^{-1}$. Additional fertilizer was given to obtain the same fertility level as on the other farms.

In 1979 the DOB-farms changed into the OBS-farms. They now fall under the authority of the Research Station for Arable Farming and Field Production of Vegetables (Proefstation voor de akkerbouw en de groenteteelt in de volle grond) at Lelystad. The purpose of the present OBS-farms is the development of three farms based on different views on the way of agronomic production in order to compare the results over a period of many years. Important aspects in this comparison are the soil fertility, the health and the yield of the crops, the quality of the products, the economic results, the use of energy and natural resources and the consequences for the environment.
The three OBS-farms are:
(1) The GA-farm (Gangbaar bedrijf = Current farm) and
(2) The GI-farm (Geïntegreerd bedrijf = Integrated farm).
Both of these farms have 17 ha of arable land and a four year's rotation. Since 1980 the crop sequence is winter wheat,

ware potatoes, spring barley/onions and sugarbeet. On the GA-farm one endeavours to maximize yields with the lowest possible costs. On the GI-farm the use of biocides and fertilizers are reduced to the minimum necessary for economic farming.

(3) The BD-farm (Biologisch-dynamisch bedrijf = Bio-dynamic farm).

This farm is operated by the principles of bio-dynamic agriculture which is based on the antroposophic philosophy of Rudolf Steiner (1949-1925). The farm is a mixed farm (22 ha) with about 20 dairy-cows, of which the manure is used on the farm. It has a ten year's rotation with 40% in ley (grass-clover). The crop sequence is ware/seed potatoes, winter wheat, winter rye, one year's ley, fodder/sugarbeet, winter wheat, oats and a three year's ley. The annual input of effective organic matter in this rotation is estimated at 2.2 tons ha^{-1} $year^{-1}$. The aim is to be self-supporting as much as possible with no application of inorganic fertilizers and biocides. Time tables are consulted for an optimal use of cosmic influences on the growth of the plants and certain special bio-dynamic preparations are applied.

The way in which the transformation of the DOB-farms to OBS-farms was carried out can be seen in fig. 3.1. About half of the KA- and WW-farm were put together as the GI-farm. The remaining halves formed the GA-farm.

The soil on the farms is a calcareous, silty clay loam. In table 3.1 soil data are presented that are based on samples taken in the DOB-period and at the start of the OBS-project. The data for the DOB-farms are averages over the years 1973-1977; the OBS data are from October 1979.

Estimations of the amounts of available nitrogen and phosphorus are made for fields in which winter wheat and potatoes were grown in the 1980 and 1981 seasons (table 3.2). In this table the assimilation of N and P_2O_5 is also given together with the ratio between the assimilation and the availability of these nutrients.

	1980						1981					
	winter wheat			potatoes			winter wheat			potatoes		
	GA	GI	BD	GA	GI	BD	GA	GI	BD	GA	GI	BD
mineral nitrogen in soil profile in February	41	69	65	10	21	93	13	33	29	103	134	53
nitrogen mineralization during growing season	80	80	70	30	30	50	70	70	70	100	100	70
nitrogen applied as fertilizer	105	105	0	310	305	0	155	105	0	300	230	0
nitrogen available from organic manure	0	0	0	0	0	60	0	0	10	40	40	50
total available nitrogen	226	254	135	350	356	203	238	208	109	543	504	173
nitrogen assimilated by the crop	180	150	80	220	160	120	180	150	80	240	160	100
ratio assimilated nitrogen/available nitrogen	0.80	0.59	0.59	0.63	0.45	0.59	0.76	0.72	0.73	0.44	0.32	0.58
water soluble phosphorus in soil profile	72	74	56	56	70	50	-	-	-	60	56	44
phosphorus applied as fertilizer	0	0	0	130	130	140[1]	0	0	0	270	270	110[1]
total available phosphorus	72	74	56	186	200	190	-	-	-	330	326	154
phosphorus assimilated by the crop	80	69	46	172	126	92	82	-	60	183	126	80
ratio assimilated phosphorus/available phosphorus	1.11	0.93	0.82	0.93	0.63	0.48	-	-	-	0.55	0.39	0.52

Table 3.2 The nitrogen (N) and phosphorus (P_2O_5) balance, made for the fields of the OBS-farms on which winter wheat and potatoes had grown in 1980 and 1981. The data are given in kg ha^{-1}.

[1] given as farmyard manure.

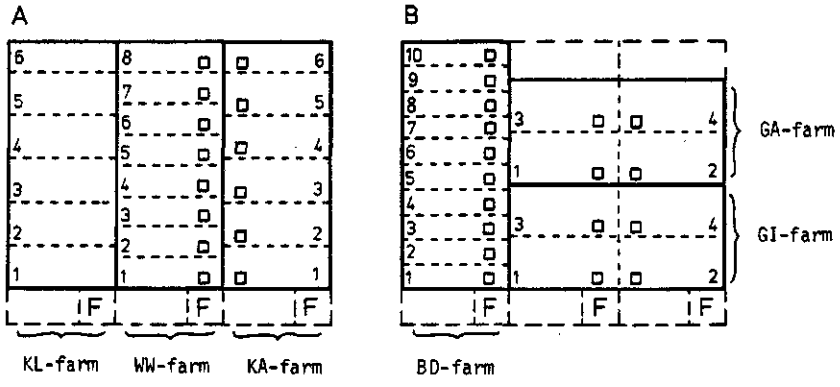

Figure 3.1 The plan of the experimental farms at Nagele.
A. The situation on the DOB-farms until 1977.
B. The farms in the OBS-project since 1979.
In 1978 the KL-farm had already adapted the rotation of the BD-farm. The KA and WW-farms changed into the GA-and GI-farms in 1979. The sampling areas are indicated in the plans (□). When a second cultivar was grown an additional sampling area was laid out in the same way.
F = Farm-house buildings.

	A			B		
farm	WW	KA	KL	GI	GA	BD
% soil particles < 16 µm	33	35	32	30	30	30
% $CaCO_3$	9	9	9	8.9	9.1	8.7
% organic matter	2.7	2.3	2.3	2.1	2.2	2.5
pH-KCl	7.3	7.3	7.4	7.5	7.5	7.5
P-Al	33	31	31	36	37	29
P_w	35	33	32	38	39	25
K-HCl	15	14	16	15	14	15

Table 3.1 Soil data of the soil on the DOB/OBS-farms.
A. Averages over the period 1973-1977 (DOB-period).
B. The data for the OBS-farms based on samples taken in 1979.
P_w gives mg P_2O_5 (water soluble) per litre soil and
K-HCl gives mg K_2O per 100 g soil.

3.3 THE LOVINK-FARM

On the Dr. H.J. Lovink-farm, which was founded in 1943 and belongs to the Institute for Soil Fertility at Haren, Groningen, a wide range of experimental fields are exploited of which the larger part was already laid out in 1944. These experiments were coded 001 through 013. Those which were used in this study on VAM deal with the effect of:

(1) Rotation

In experiment 001 four rotations are compared viz.: (I) continuous winter wheat, (II) a rotation of winter wheat, winter wheat, potatoes, winter wheat, winter wheat, sugarbeet, (III) a rotation of winter wheat, sugarbeet, winter wheat, potatoes and (IV) a rotation of winter wheat, sugarbeet, oats and potatoes.

(2) Green manure

In experiment 004 a rotation of potatoes, winter wheat, sugarbeet and spring barley is interrupted by the cultivation of a green manure crop every four years. As green manure crops are grown a grass-clover ley (II), red clover (III), lucerne (IV) and a control without green manure (I). In the year that the green manure crops are grown, oats are grown on the control plots. In recent years, the green manure crops were grown in 1976 and 1980. The experiment is performed in four replicates. Except for the year in which the green manure crops are grown, each of the four rotation crops is present in every treatment. In the "green manure year" the four replicates of the control plot each have a different cropping history. Fig. 3.2 shows the basic plan of this experiment.

(3) Different farming systems (mini-DOB experiment)

This experiment 006 is a replication of the DOB-farms as described under 3.2, but on a small scale. The treatments on these mini-DOB-farms are:

(I) An object comparable with the KA-farm in which no organic manure is used. The rotation is winter wheat, flax, seed potatoes, sugarbeet, spring barley and ware potatoes.

(II) An object on which every three years two times a green manure crop of Italian rye grass or white clover is grown as

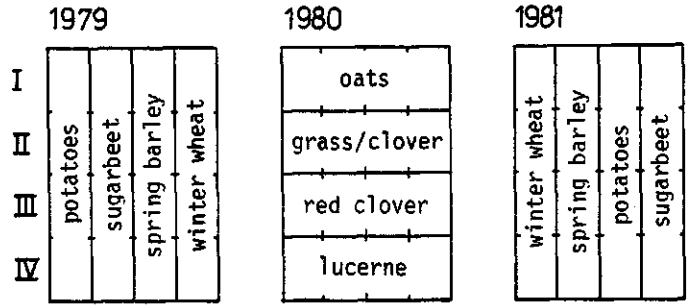

Figure 3.2 The plan of the green manure experiment on the Lovink-farm (004) over the period 1979-1981. The Roman figures indicate the green manure treatments. The green manure crops were grown in 1980. The other crops are grown in a rotation of winter wheat, sugarbeet, spring barley and potatoes.

undercrop. This object is comparable with the KL-farm and has the same rotation as object I.
(III) An object comparable with the WW-farm and with the use of farmyard manure or compost (35 ton ha^{-1} $year^{-1}$). There are green manure crops and leys in the rotation. This rotation is winter wheat, flax, one year's ley, seed potatoes, sugarbeet, spring barley, one year's ley and ware potatoes.
In all the three objects the nitrogen fertilizer on winter wheat is partly given as a top dressing in the growth stage Feekes 6-7.

(4) Soil tillage

In experiment 007 the effect of different degrees of soil tillage on crop productivity is studied. The three main tillage variants can be described as traditional (I), intermediate (II) and minimal tillage (III). The detailed tillage practices are given in table 3.3 together with the rate of fertilizer applications. The rotation is winter wheat, potatoes, and sugarbeet.

(5) Farmyard manure

In experiment 010 the effect of the additional application of farmyard manure (FYM) on crop productivity is compared with that of the use of mineral fertilizers only. The FYM

Tillage	potatoes		winter wheat		sugarbeet	
minimal	- plough (12-15 cm)		- cultivator (shallow, 2×)		- plough (12-15 cm)	
intermediate	- cultivator (20 cm)		- cultivator (shallow, 2×) - cultivator (12-15 cm)		- cultivator (25 cm)	
traditional	- plough (20 cm)		- cultivator (shallow, 2×) - plough (12-15 cm)		- plough 25 cm)	
fertilizers	1979	1980	1979	1980	1979	1980
kg P_2O_5 ha^{-1}	108	108	108	108	54	54
kg N ha^{-1}	132[1]	171[1]	93[1]	117[2]	109[1]	170[2]
kg K_2O ha^{-1}	270	270	0	0	120	120
organic manure (t ha^{-1})	0	0	crop residues of sugarbeet		22 champost[3]	20

Table 3.3 The main tillage practices, carried out prior to crop growing, in the soil tillage experiment on the Lovink-farm and applied fertilizers. The crop rotation was winter wheat, potatoes and sugarbeet. VAM development has been assessed in winter wheat.

[1] nitrogen fertilizer given in the form of calcium nitrate.

[2] nitrogen fertilizer given in the form of ammonium nitrate.

[3] Champost = spent mushroom compost.

is applied at an amount of 20 tons ha^{-1} every other year from 1946 onward. The phosphorus and potassium contained in the FYM is compensated for in the control treatment by additional fertilizers. During the period 1977-1981 the rotation was seed potatoes, spring barley, ware potatoes, winter wheat and sugarbeet and FYM was applied in 1978 and 1980.

(6) Phosphorus availability

Experiment 012 is carried out on a field that consists of a number of plots with different levels of phosphorus. These plots are regularly fertilized with various amounts of phosphorus (0, 250 and 500 kg P_2O_5 ha^{-1} in the form of super

phosphate). This results in a wide range of levels of available phosphorus. All the plots receive the same amount of nitrogen fertilizer. During the period 1977-1981 the rotation was spring barley, ware potatoes, winter wheat, sugarbeet and spring barley.

In most of the above mentioned experiments different nitrogen levels were applied. Until 1981 nitrogen was mainly given in the form of calcium nitrate, but from 1981 onward ammonium nitrate is used. The amounts of other plant nutrients were sufficient for optimal growth.

The soil on the Lovink-farm is a calcareous clay loam with about 30% of the soil particles <16 μm, 10% $CaCO_3$, 2.3% organic matter and a pH of about 7.5. This is very similar to the soil on the DOB/OBS-farms at Nagele.

4 *THE VAM SITUATION ON THE DOB/OBS-FARMS*

4.1 INTRODUCTION

The investigations described in this chapter started in the autumn of 1978. The first question to be answered was about the relative presence of VAM fungi in the fields of the three farms. In order to obtain information about the VAM situation in the last year of the DOB-system, soil samples were taken and analyzed for numbers of VAM fungal spores. This would make it possible to compare the effect of the three farming systems on VAM.

It turned out to be possible to study the presence of spore populations of VAM fungi in previous years by analyzing old stored soil samples. These had originally been taken for chemical soil analysis. The oldest samples were of 1951.

As explained in chapter 3 the DOB-project was changed into the OBS-project and in this chapter the VAM situaton in the first years of the new OBS-project is also presented. The investigation of VAM on the OBS-farms is mainly based upon estimations of mycorrhizal development in winter wheat and potatoes. In addition, experiments concerning the effectivity of the VAM fungi, the VAM inoculum potential and the relative frequency of the different spore types encountered on the DOB/OBS-farms were carried out.

4.2 MATERIALS AND METHODS

4.2.1 *Collecting soil samples*

Soil samples were taken using a soil auger with a diameter of 6 cm and a core length of 20 cm. From each field eight soil cores were collected in an area of about 30 x 30 meters. These sampling areas were situated in the field as indicated in chapter 3 (fig. 3.1). Soil samples used for the estimation of the number of spores and for the isolation of VAM fungi

were taken on the DOB-farms in September 1978. The soil samples for collecting roots from the winter wheat crop were taken with the wheat plant(s) in the centre of the core. In the potato crop each sample consisted of two soil cores taken at opposite sides of the plant, 10 cm from the stem basis. The samples in the winter wheat crop were taken during the flowering period, in June, those in the potato fields during the period that the highest VAM infection was expected. This latter was, as indicated by Black & Tinker (1977), in the middle of July.

The soil used for estimating the VAM inoculum potential and the spore type frequency was collected with a small garden shovel, samples being taken at random up to a total weight of about 6-8 kg of soil. This sampling was done in April 1981. Another sampling for the assessment of spore type frequencies was done in September 1981. In 1981 the potato roots on the GA-farm were sampled outside the sampling area because the larger part of the crop had already been harvested. The soil samples were stored in plastic bags at 4°C until further elaboration.

The soil samples that have been used for the study of the VAM situation during the previous DOB-period have been stored dry at the Institute for Soil Fertility at Haren and were kindly placed at my disposal.

4.2.2 *Estimating the numbers of spores*

Three different methods were used to recover spores of VAM fungi from the soil.

(1) The wet sieving and decanting method. This method, as described by Gerdemann & Nicolson (1963) was used for the recovery of spores of VAM fungi from the soil samples taken in September 1978. The eight soil samples from each sampling area were mixed and air dried and three subsamples of 10 g soil were taken from it. Each subsample was suspended in water, decanted three times and sieved into four fractions: 250 µm, 250-150 µm, 150-106 µm and 106-75 µm. The different fractions were collected on filter paper and the spores were counted by scanning under a dissecting microscope (25-50x).

(2) The centrifugal flotation technique. This technique is primarily based on the description by Ohms (1957) using centrifugation in a sucrose solution in order to separate spores from the light and the heavy particles of the soil. The method was modified as follows:
Ten gram of soil (on dry weight basis) was suspended in water and sieved in fractions to facilitate the sieving. Afterwards the fractions from 250 to 75 µm were put together and were suspended in a wide mouth centrifuge tube (33 mm in diameter), containing 45 ml of a solution of 2N sucrose + 2% Calgon (sodium polyphosphate). The suspension was centrifugated and then filtered through a membrane filter. The spores were counted using a dissecting microscope. The soil samples that were taken during the previous DOB-period were examined by this method.
(3) The fluidising column method. This method has been described by Trudgill et al. (1972) as a method to extract nematodes from soil. In the apparatus of Trudgill, a 250-106 µm sieve fraction from a 100 g sample of soil (on dry weight basis) was fractionated by a 7 $l.h^{-1}$ water stream during one hour. The light fraction that overflowed, was collected on a sieve and contained most of the spores. It was resuspended in 50 ml of water. From both suspensions two aliquots of 10 ml were taken by mixing the suspension with an air stream. These samples were examined for spores of VAM fungi using a dissecting microscope. Only spores that seemed to be alive were scored and classified.
The sieve fraction of 250-355 µm and the material that retained on the 355 µm sieve was examined directly without using the apparatus. All the spores larger than 106 µm were recovered. The soil samples were fractionated only to facilitate the scanning of the spores. This method was used for estimating the relative frequency of the various spore types.

4.2.3 *Assessment of VAM*

The soil samples that were used for collecting roots were saturated with a solution of oxalic acid in water to facilitate the root washing (Heringa et al., 1980). The samples

were kept 30-60 minutes in this solution. After washing in water the roots were cut into pieces of 3-5 mm and preserved in FAA fixative[1] until the staining procedure. A sample of 0.5-1.0 ml was then stained by a method based upon that of Phillips and Hayman (1970) and which consisted of the following steps:

- heating the roots in 10% KOH at 90 °C (wheat roots one hour, potato roots about 1.5 hour);
- two times washing with tap water;
- acidifying with 0.1 N HCl;
- two times washing with tap water;
- staining in 0.05% trypan blue in lactophenol for 5-15 minutes;
- clearing of the roots.

In the beginning lactophenol was used for clearing, later on tap water was used which gave satisfactory results. After clearing, the roots were stored in glycerol. For the determination of the percentage VAM infection the line intersection method was used (Ambler & Young, 1977). For this quantification of the VAM infection a glass slide was used with a surface area of 95x56 mm. Around the edge a 7x0.3 mm plastic tape barrier was applied on which rested a cover glass of 90x50 mm. The stained sample was put on the slide and the percentage of VAM was scored under a compound microscope. This scoring was based on the mycorrhizal status of the roots at the intersections of these roots with a fixed point in the ocular of the microscope. Per sample 250-300 intersections were scored.

4.2.4 *Testing the inoculum potential*

The soil samples that were used for estimating the numbers of diaspores that can result in mycorrhizal symbiosis were slightly dried and sieved through a screen with a 2 mm mesh. A part of each sample was autoclaved (1.5 hour, 121 °C) and used to make dilution series with the unsterilized part of

[1] Formalin 35% - Acetic acid (glacial) - Alcohol 50% as 13:5:200.

the soil sample. A dilution series from 4^{-2} to 4^{-7} was made for each soil. From each soil ten plastic pots (5.5 cm in diameter) were filled with 50 g soil (on dry weight basis) and in each pot three seedlings of *Trifolium pratense* cv. Barfiola were planted. They were inoculated with *Rhizobium trifolii* strain K 8[1]. After 8-11 weeks the roots of the plants were carefully washed free from soil. The whole root systems were stained (see 4.2.3) and examined for mycorrhizal infection using a dissecting microscope. The most probable number was calculated from the results by means of the Gumble distribution

$$\{F(x) = e^{-e^{-x}},\ -\infty < x < \infty\}.$$

4.2.5 *Collecting isolates*

Different spore types were isolated by means of the wet sieving and decanting technique (see 4.2.2) from soil samples that were taken on the DOB-farms in September 1978. A rough selection was made on the basis of morphological spore characters visible at low magnification under a dissecting microscope. Small groups, consisting of 3-6 spores which appeared identical, were used for inoculation to the roots of tomato plants (cv. Moneymaker). From successful inoculations monospore cultures were then made. These cultures which were identified with the help of Dr. C. Walker, Edinburgh, were used in further experiments.

4.2.6 *Effectivity of the VAM fungi*

(1) The effectivity of the monospore isolates.

The growth stimulating effect of VAM fungi isolated from soil of the DOB-farms (see 4.2.5) was tested with *Trifolium pratense* cv. Barfiola on a nutrient deficient soil (see for data table 6.1, soil B). Two isolates from other origins and two different controls (with living uninfected roots and without inoculum) were used for comparison. The two reference

[1] From Dept. for Microbiology, Agricultural University, Wageningen.

isolates were one of *Glomus mosseae* originally obtained from Dr. B. Mosse in 1977 and one obtained from a soil similar to the above mentioned nutrient deficient soil and identified by Dr. C. Walker as belonging to a not yet formally described species viz. *Glomus occultus*. The isolates from the DOB-farms are described in 4.3.3 and summarized in figure 4.1. In preliminary experiments red clover cv. Barfiola turned out to be a suitable host for this kind of experiments as it responded strongly to VAM. The experiment was performed with 700 g of soil (on dry weight basis) in plastic pots of 12 cm in diameter. The soil was kept at about 60 percent of the upper plasticity limit by weighing. The soil surface was covered with a layer of fine gravel to reduce evaporation and to obtain an equal distribution of the moisture in the pot. During the experiment the position of the pots was changed every time when the pots were weighed. The experiment lasted nine weeks in the greenhouse with natural light and a temperature between 20 °C and 24 °C (occasionally up to 30 °C). The inoculum of the different isolates consisted of infected roots from about three months old tomato plants. The quantity of the inoculum used was based on the degree of VAM infection in the roots in such a way that the product of the percentage VAM and fresh weight of the roots was equal to 20. This amounted to between 0.33 and 0.63 g fresh root inoculum per pot. The inoculum was spread at a level of 4 cm below the surface of the soil. In the first control (I) 0.5 g uninfected tomato roots were used. In each pot one red clover seedling (about ten days old) was planted and inoculated with *Rhizobium trifolii* strain K 8.

(2) The effectivity of the VAM population in field soil.

In February 1981, soil samples were collected (about 8 - 10 kg taken at random at the three OBS-farms) from the fields which had grown crops of winter wheat (GA- and GI-farm) or spring wheat (BD-farm field 5) in 1980 and sugarbeet in 1979. Samples were also collected from a field of the BD-farm (field 9) which was in ley in 1980 and 1979. Before further elaboration, the soil was dried at room temperature until sieving with a coarse sieve (4 mm) became possible without damaging

the soil structure too drastically. A few days later the soil samples were sieved again. This time a 2 mm sieve was used and the samples were divided in two equal parts. One part was γ-irradiated (2.5 Mrad) to kill the native VAM fungi in the soil with as little damage as possible to the chemical and physical status of the soil. Since all the micro-organisms are killed by irradiation and nutrients from those dead organisms become available again, the sterilized soils were inoculated with filtered suspensions of unsterilized soil. It was assumed that the inoculated micro-organisms would assimilate the extra nutrients available before the test plants (red clover cv. Barfiola) were planted. The suspensions were derived by suspending 100 g of unsterilized soil in 200 ml of water. This mixture was filtered through cotton wool, then through filter paper and finally through a 8 μm millipore filter. The filtrate was added to the sterilized soil which was subsequently kept in an aerated plastic bag for four weeks. The pots (12 cm in diameter) were filled with 650 g of soil (on a basis of dry weight) 18 days prior to planting. The red clover plants, that have been inoculated with *Rhizobium trifolii* strain K 8 were grown under the same conditions as described above. After nine weeks the dry weight of the shoots was measured.

4.3 RESULTS

4.3.1 *VAM spores present in the DOB soils*

The number of spores that were found in the soil samples taken in September 1978 on the three farms are given in table 4.1. After winter wheat and potatoes the largest total number of recovered spores was found in the soil from the WW-farm. The smallest number was found in the soil from the KA-farm. No sporocarps were found. Noteworthy is the large difference in the 106-75 μm fraction between the KA-farm and the other farms. This fraction however, contained many sporelike bodies of which it could not clearly be established whether they were mature spores or only immature spores and vesicles. It

	potatoes			winter wheat		
farm	WW	KL[1]	KA	WW	KL[1]	KA
previous crop[2]	G	F	B	P	B	P
sieve fraction						
>250 µm	0	0	0	0	0	0
250-150 µm	35	26	25	49	27	18
150-106 µm	121	126	83	121	103	109
106- 75 µm	218	181	113	180	179	126
mean of total	374[a]	333[a]	221[b]	350[x]	309[x]	253[y]
s.d.	24	17	28	33	31	21

Table 4.1 Numbers of VAM spores recovered by wet sieving in four sieve fractions from ten gram soil of the three DOB-farms. The data are means of three subsamples. Sampling date: September 5th 1978.

[1] In 1978 the KL-farm had already adapted the rotation of the BD-farm in the OBS-project.

[2] G = pasture; B = spring barley; F = flax; P = potatoes.

The values with the same letter are not significantly different ($p<0.05$; t-test).

was not possible to determine the viability of all the individual spores, therefore all the spores and sporelike bodies were counted. It was estimated that probably 70-90% of the recovered spores were non-viable. This assumption is based on inoculations of the sporelike bodies to tomato plants that never gave any infection.

Although significant differences were found between the number of spores, no definite conclusions about the effect of the different types of farming on the presence of VAM spores could yet be drawn. This was not done on the basis of the results of the spore counts that were made in the soil samples taken over the previous DOB-period. These results are given in table 4.2. In this table the number of spores are recorded that were found in the soil after a wheat crop. An exception was made for the first soil samples that were taken on the DOB-farms in 1951. A large variance was found in the spore counts. For not any of the farms a significant regression line could be calculated from the number of spores. Even for all the spore counts on the three farms together no increase or decrease was found over the previous DOB-period.

year		1951	1957	1960	1965	1966	1974	1975	1976	1977
sampling date		19-9	22-2	23-3	31-5	18-5	2-5	26-5	4-5	18-5
KA-farm	field 2	326	259		262					296
	field 4	359		375				202		
	field 5	237							307	
WW-farm	field 5	300	156						260	
	field 7	380		392		276	302			
KL-farm	field 2	383	317		308					338
	field 4	266		259				370		

Table 4.2 Numbers of VAM spores recovered from ten gram soil, sampled during the previous DOB-period. In the year prior to sampling, a winter wheat crop was grown. Except for 1951, the first year of the DOB-farms.

The mean number of spores was 292 per 10 g of dry soil with an upper and lower limit of 392 and 156 spores respectively. Samples taken in the period 1957-1965 after a flax crop resulted in an average of 381 spores per 10 g of dry soil (s.d. = 86, n = 6). This tended to be more than after winter wheat.

From these results it is concluded that 26 years of crop growing at three different input levels of organic material had no significant effect on the numbers of VAM spores in the soils of the DOB-farms. Only on the KL-farm a slight tendency toward increased spore counts was found, although this was not statistically significant.

4.3.2 *VAM development in the roots*

In the three successive years (1979-1981) the mycorrhizal status of winter wheat and potatoes was examined on the three OBS-farms. The results of this investigation are presented in table 4.3. The VAM development is given as percentage root length infected.

On the BD-farm the VAM infection in winter wheat cv. Manella increased from 42% in 1979 to 59% in 1981 and was significantly more than on the GA- and GI-farm. On these latter two farms it fluctuated around 15 to 20%. In 1980 the results

season	winter wheat						potatoes						
	farm	cultivar	previous crop[1]	% VAM	s.d.	yield (t ha^{-1})	farm	cultivar	previous crop[1]	% VAM	s.d.	yield (t ha^{-1}) total	yield (t ha^{-1}) >35 mm
1979	GI	Nautica	P	23.7^{a}	9.2	5.8	GI	Bintje	L	12.9^{a}	3.3	50.3	45.5
	BD	Manella	P	42.3^{b}	6.4	4.5	BD	Irene	L	12.3^{a}	4.2	23.3	20.3
1980	GA	Nautica	SB	14.8^{a}	6.0	6.9	GA	Bintje	SW+G	9.5^{a}	2.1	54.7	49.1
	GA	Arminda	SB	24.7^{b}	6.1	7.4							
	GI	Nautica	SB	12.3^{a}	6.4	6.2	GI	Probaat	WW	6.7^{b}	2.0	41.0	37.2
	GI	Arminda	SB	16.6^{a}	6.8	6.4							
	BD	Arminda	P	49.1^{c}	7.4	4.7	BD	Irene	L	18.4^{c}	5.2	29.8	21.6[2]
	BD	Manella	P	46.5^{c}	5.2	4.6	BD	Briljant	L	14.3^{c}	2.8	33.7	
1981	GA	cv. mix[3]	SB	17.6^{a}	4.0	7.3	GA	Irene	WW	3.8^{a}	1.4	55.8	52.6
	GI	cv. mix	SB	19.8^{a}	3.9	6.4	GI	Irene	WW	4.8^{a}	1.6	27.6	
	BD7	cv. mix	SB/FB	48.9^{b}	4.7	4.0	BD	Irene	L	16.0^{b}	3.5	25.2	23.5
	BD2	Manella	P	58.8^{c}	3.7	5.1							

Table 4.3 VAM development in, and the yield of winter wheat and potatoes grown at the OBS-farms during the period 1979 - 1981. The % VAM is given as a mean of eight replicates. The values with the same letter are not significantly different ($p<0.05$; t-test). Comparisons are made within the same growing season.

[1] P = potatoes; L = ley; SW = spring wheat; WW = winter wheat; G = green manure crop; SB = sugarbeet; FB = fodderbeet.

[2] exclusive 3.9 t ha^{-1} seed potatoes 28-55 mm.

[3] cv. mix = cultivar mixture of Anouska, Arminda and Nautica.

showed also a slight difference between the cultivars Arminda and Nautica. The leafy Arminda tended to be more infected than the cultivars Nautica and Manella. This comparison could not be made in 1981 because of the use of cultivar mixtures. On the BD-farm the Manella cultivar was grown also. This is a cultivar that is assumed to be very suitable for bio-dynamic farming.

Because the farms have different rotations, comparisons were not always easy to make. In this respect it is noteworthy to take the previous crops into account. On the BD-farm potato, a mycorrhizal plant, was grown before the winter wheat. On the GA-farm sugarbeet, a nonmycorrhizal plant, was grown before the winter wheat. This undoubtedly influenced the inoculum density for the 1981-season as will be indicated in 4.3.4. In 1979, the first year of the OBS, the winter wheat had potatoes as a previous crop on the BD-farm as well as on the GI-farm. In that year on the GI-farm, the VAM development in the Nautica cultivar tended to be better than in 1980 when the same cultivar was grown after sugarbeet. The nutritional status of the soil, which was not measured in 1979, can also have been the reason for the difference. In 1980 and 1981, the plant nutrients in the soil and especially the amount of available nitrogen was much larger on the GA- and GI-farm than on the BD-farm (see table 3.2). The N-availability plays an important role in the VAM development in winter wheat as will be shown in chapter 5. In the same two years there was not much difference in the amount of available phosphorus and this P-availability did probably not account for the differences in the infection levels in the wheat. The efficiency of the N-assimilation and P_2O_5-assimilation, given in table 3.2 as the ratio assimilated/available, showed no clear correlation with the level of VAM infection (table 4.3). It tended even more to a negative correlation: the better the nutritional status of the soil the better the efficiency of the N- and P_2O_5-assimilation. Using all the winter wheat data of the three years a negative correlation ($r = -0.83$) was found between the percentage of VAM and the yield.

In the potato crop the VAM development was much less than in winter wheat. In 1979 there was no difference at all between the VAM infection on the BD-farm and the GI-farm. On both farms a ley was the previous crop. In 1980 and 1981 however, only on the BD-farm the previous crop was a ley. In these years a difference between the farms was found. In spite of the large differences in the amount of available nitrogen the VAM infection in potato on the BD-farm did not exceed very much the VAM infection levels found in potato on the other farms.

4.3.3 *Spore types and their frequency*

(1) The spore types.

In the DOB/OBS soils four different spore types, a, b, c and d, were found.

Type a was an isolate consisting of many freely formed chlamydospores. Sporocarps are seldom formed in pot culture. The yellow spores had a thin wall, a funnel shaped attachment hypha and were clearly vacuolated. Most of the spores float on water. No vesicles are found in infected roots. According to the classification of Gerdemann & Trappe (1974) this spore type resembled most *Glomus mosseae*. This type was isolated from the WW- and the KA-farm.

Type b differed from type a in the formation of many sporocarps with up to 32 spores per sporocarp. Free chlamydospores were also found, but they did not float on water. The other characters were very much the same. There is also no vesicle formation in the roots. It was identified as *Glomus mosseae*. This type was isolated from the KL-farm.

A remarkable feature of spore type c is its thick spore wall and the streight subtending hypha with its wall thickening near the spore. No formation of sporocarps and no vesicles in the roots were found. The spore production was less abundant than with *Glomus mosseae*. Determinated as *Glomus macrocarpus* var. *geosporus*. This type was isolated from the WW- and KL-farm.

Type d distinguished itself from *G. mosseae* by the formation of one-, sometimes two- or three-spored sporocarps only. The

freely formed chlamydospores do not differ from the chlamydospores of *G. mosseae* (type b). There was no formation of vesicles. This type was identified as *Glomus monosporus* and was isolated from the WW-farm.

(2) Frequency of the spore types.

For quantifying these spore types in a field soil it was necessary to make a simplification because it was not possible, for practical reasons, to isolate every spore and to identify it according to the usual methods. For instance, no distinction could be made between *Glomus mosseae* and *G. monosporus*. This latter species was scored on the occurrence of one-spored sporocarps only. Also between the two *G. mosseae* types no distinction was made because the difference between the chlamydospores only was too small for a clear identification (cf. Abbott & Robson, 1979).

The classification "unknown" consisted of spores that could not clearly be classified in any one group. This was a heterogeneous group without any one type being particularly prevalent. The observations on the frequency of the spore types are summarized as follows:

- April 1981 (table 4.4 A).

The highest spore numbers were found in the soil of the BD-farm. On the GA- and GI-farm more spores were recovered after winter wheat than after sugarbeet.

In the same samples, the spores of *G. macrocarpus* var. *geosporus* were found to be dominant in most of the plots on the three farms. *G. monosporus* was found with a low frequency except on the GI-farm after sugarbeet. In this particular situation its relative frequency was 0.28.

- September 1981 (table 4.4 B).

During the growing season some changes in the population of the spores took place. It was indicated that there had been an increase in the number of spores under winter wheat and a decrease of those under potatoes. In the winter wheat fields the dominance of *G. macrocarpus* var. *geosporus* had been taken over by *G. mosseae*. An increase in the number of spores was found for *G. monosporus* under winter wheat. After the growth of a potato crop *G. macrocarpus* var. *geosporus*

A. April 1981

farm	crop	previous crop	Glomus mosseae		Glomus macro-carpus		Glomus mono-sporus	Unknown	Total number of spores
			n[1]	f[2]	n	f	n	n	n
GA	WW	SB	10	0.20	27	0.54	5	8	50
GA	P	WW	28	0.34	38	0.48	3	10	79
GI	WW	SB	13	0.21	23	0.38	17	8	61
GI	P	WW	20	0.21	65	0.68	0	10	95
BD	WW	SB/FB	53	0.44	43	0.36	9	15	120
BD	WW	P	23	0.21	63	0.57	6	18	110
BD	P	L	56	0.42	63	0.47	0	15	134

B. September 1981

farm	crop	previous crop	Glomus mosseae		Glomus macro-carpus		Glomus mono-sporus	Unknown	Total number of spores
			n[1]	f[2]	n	f	n	n	n
GA	WW	SB	53	0.35	55	0.36	16	30	153
GA	P	WW	10	0.18	30	0.55	7	8	55
GI	WW	SB	33	0.46	13	0.18	18	8	72
GI	P	WW	15	0.24	43	0.69	1	3	62
BD	WW	SB/FB	105	0.56	25	0.13	25	33	188
BD	WW	P	120	0.59	55	0.27	15	13	203
BD	P	L	15	0.54	5	0.18	3	5	28

Table 4.4 Numbers of viable VAM spores (> 106 µm) recovered from soils of the OBS-farms. Samples were taken at the beginning of the growing season (A) and after maturing of the crops (B) on the same fields.
The numbers of spores are calculated on the basis of 100 g dry soil.
[1] n = number of spores
[2] f = relative frequency of the spores
See for other abbreviations page 6.

was still the most frequently recovered spore type and dominated over *G. mosseae* even more than in April, except on the BD-farm. On this latter farm the total number of spores was much lower than in April.

A. winter wheat 1981

farm	cultivar	previous crops		number of diaspores per 100 g soil (April)	spores > 106 µm per 100 g soil (April)	% VAM (June)
		1980	1979			
GA	cv. mix	SB	ON/B	299	50	17.6
GI	cv. mix	SB	ON/B	212	61	19.8
BD	cv. mix	SB/FB	L, 1st year	460	120	48.9
BD	Manella	P	L, 2nd year	1395	110	58.8

B. potatoes 1981

farm	cultivar	previous crops		number of diaspores per 100 g soil (April)	spores > 106 µm per 100 g soil (April)	% VAM (July)
		1980	1979			
GA	Irene	WW	SB	713	79	3.8
GI	Irene	WW	SB	400	95	4.8
BD	Irene	L	L, 2nd year	1956	134	16.0

Table 4.5 Comparison between the number of VAM diaspores, the recovered VAM spores and the subsequent VAM infection in winter wheat (A) and potatoes (B) at the OBS-farms.
See for abbreviations page 6.

4.3.4 *Inoculum potential*

The results of the estimation of the inoculum potential on the OBS-farms are presented in table 4.5. At a confidence level of 90% the most probable number of VAM diaspores (MPN) was defined as lying between 0.62 EN and 1.61 EN (EN = the estimated number of diaspores).

The highest inoculum potentials were found on the BD-farm. They were especially high on those fields which had been in ley for the previous two or three years. The lowest EN of diaspores were found on the GA- and GI-farm after the sugar-beet crop. On the same farms but after winter wheat intermediate results were obtained. Comparison of these results with those of the spore numbers found in the same soil (cf. 4.3.3) shows that the numbers of spores reflect the same tendency as the EN of diaspores.
However, the spore numbers represented only 7 to 29 percent of the estimated numbers of diaspores. This was for example

7.9 percent for the winter wheat field, cv. Manella, on the BD-farm (in 1978 and 1979 in ley) and 6.9 percent for the potato field, cv. Irene, on the BD-farm (1978, 1979 and 1980 in ley). In July only low degrees of VAM infection were found in the potato fields despite of the relatively high estimated numbers of diaspores in these fields at the beginning of the growing season.

4.3.5 *Effectivity of the VAM fungi*

(1) The effectivity of the monospore isolates.

All the DOB-isolates (isolate number 1-6) that were tested had a positive effect on the growth of red clover (fig. 4.1). Only *G. macrocarpus* var. *geosporus* (isolate 1) gave a small effect. The effect of the other five DOB-isolates did not differ very much from that of the *G. mosseae* strain of Dr. B. Mosse. The *G. occultus* isolate induced the best response. Inoculation with non-infected living tomato roots gave no effect.

isolate	origine	type[1]
1 G. macrocarpus	KL	c
2 G. mosseae	KL	b
3 G. macrocarpus	WW	c
4 G. mosseae	WW	a
5 G. monosporus	WW	b
6 G. mosseae	KA	a
7 G. mosseae	B. Mosse	
8 G. occultus	Lelystad	
9 control I		
10 control II		

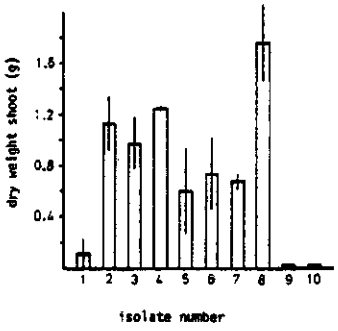

Figure 4.1 The effectivity of different isolates of VAM fungi as tested with red clover (nine weeks of growth) in nutrient deficient soil (soil B, see 6.2). Each isolate was inoculated as infected tomato roots; the same total infected root length per isolate. In control I uninfected tomato roots were used; in control II no roots were used at all.

[1] the types refer to the description as given in 4.3.3.

farm-field	previous crops			dry weight shoots (g)		flowering[2]
	1979	1980		mean	s.d.	
BD-9	L	L	- VAM[1]	4.96	0.30	+
			+ VAM	4.83	0.37	+
BD-5	SB	SW	- VAM	3.49*	0.85	-
			+ VAM	2.38	0.52	-
GI-3	SB	WW	- VAM	5.84*	0.60	+
			+ VAM	4.64	0.86	±
GA-3	SB	WW	- VAM	5.37*	0.64	+
			+ VAM	3.70	0.49	±

Table 4.6 Effect of soil sterilization on the growth of red clover (nine weeks of growth) in soil from the OBS-farms.

[1] - VAM is equivalent to sterilized soil.
+ VAM is equivalent to unsterilized soil.

[2] The flowering of the plants indicates the stage of plant development.

See for abbreviations page 6.

The asterisks (*) indicate significant differences between + and - VAM ($p<0.05$; t-test).

(2) Effectivity of the VAM population in field soil.

The results, presented in table 4.6, gave no clear answer. Except for the soil of field 9 on the BD-farm, the red clover plants grown on the unsterilized soil (+ VAM) were significant lower in shoot dry weight than the plants grown in sterilized soil. The smallest plants were still bigger than the plants inoculated with the best monospore isolate from the DOB-farms as described above.

4.4 DISCUSSION

The establishment of VAM fungi in the North East Polder had already taken place before in 1951 the first soil samples were taken that were examined for spores of VAM fungi. The numbers of spores found in that particular year did not differ much from those found in recent years. How these fungi invaded into the polder, which fell dry in 1942, is a matter of speculation. The main way of dispersal is according to Gerdemann & Trappe (1974) by transport of soil and by infected plants. Worms, wasps and birds can spread spores (McIlveen & Cole, 1976) and also spores from the faeces of rodents were

able to establish mycorrhizas (Trappe & Maser, 1976; Rothwell & Holt, 1978). After their introduction into the polder it will have taken some time before the VAM fungi had become fully established. This would also correspond with the observations done in the more recent reclaimed polders in which, in the first years of crop growing, clover, green peas and flax grew poorly in soil with a moderate phosphorus level. Cereals and rape seed had no problems and gave normal yields (Ir.G.J. de Jong, RIJP, pers. comm.). This may indicate the lack of VAM in the first years of the polders.

The numbers of spores found in the soil sampled on the three farms over the previous DOB-period showed much variation. The results obtained in 1978 should therefore be regarded with caution. The nutrient availability differed very little on these farms (table 3.1). The only difference was in the organic matter content of the soil, although, this difference was small. Older work (Johnston, 1949) showed more VAM infection in soils of comparatively high organic matter status. This was not confirmed on the basis of spore counts in the case of the DOB-farms. The effect that organic matter can have on VAM development will be discussed in further detail in 5.4.

The VAM fungal species found were all *Glomus* species, predominantly *G. mosseae*. This species seems to be associated with rich, fine textured high pH soils (Abbott & Robson, 1977; Mosse & Bowen, 1968; Khan, 1974 and Powell, 1977) and this is in agreement with the soil on the DOB-farms. Most of the VAM fungi isolated from the phosphorus rich DOB-farm soils appeared to be effective in the symbiosis with red clover in nutrient deficient soil. Whether this means that these fungi are adapted to these fertile soils without having lost their effectiveness or that they are of importance, even in rich soils, is still uncertain. Adaptation of VAM fungi to the soil seems to occur (Lambert et al. 1980).

In 1979, the VAM development in winter wheat in the newly started OBS-project was already significantly larger on the BD-farm than on the GI-farm. In 1980 and 1981 the difference between the GA- and BD-farm and between GI- and BD-farm became

more pronounced. The main factors that probably accounted for this difference are:
(1) The amount of available nitrogen. As will be described in chapter 5 and 6, differences in the amount of available nitrogen can markedly influence the VAM development in winter wheat. In table 3.2 it is shown that the estimated amount of available nitrogen was much lower on the BD-farm than on the GA- and GI-farm.
(2) The VAM inoculum potential. If potatoes are not considered as good VAM hosts (the number of VAM spores tended to decrease during the growth of this crop (table 4.4)), 80% of the crops in the rotation on the BD-farm and only 50% in the rotation of the GI- and GA-farm are good VAM hosts. This undoubtfully leads to a better build-up of the VAM inoculum potential on the BD-farm and this results in a more rapid VAM development.
(3) The root growth. Although there is no direct evidence available yet for the influence of this factor, the slow development of the crop on the BD-farm in the first period of the growing season may indicate a slow growth of the roots. This can give an increased percentage of VAM in the roots (see 7.3.1).

Except for the nitrogen, no other nutrient deficiencies occurred in the winter wheat crop on the BD-farm.

In the potato crop only a slight, but significant difference was found between the BD-farm and the other two farms. Deficiencies other than that of nitrogen, like a phosphorus deficiency, have to prevail to arrive at higher levels of VAM infection in this potato crop.

Notable was the shift of the dominance of *Glomus macrocarpus* var. *geosporus* into a dominance of *G. mosseae* after a season of wheat growing (table 4.4). This did not happen after growing potatoes. In this latter case, even the total number of VAM spores tended to decrease and the relative frequency of *G. macrocarpus* var. *geosporus* increased. Nemec et al. (1981) found relative large numbers of *Glomus macrocarpus* spores in citrus orchards with high levels of phosphorus in the soil. The relative large numbers of this fungus in the

potato fields (except on the BD-farm) are probably more related with a limited VAM development in potatoes and a larger ecological persistance of the thick walled chlamydospores of this species than that it is indicating a preference of *G. macrocarpus* var. *geosporus* for soils high in phosphorus. After a season of wheat growing, the number of *G. mosseae* spores had been increased more than the number of *G. macrocarpus* var. *geosporus* spores.

No evidence was obtained to illustrate an effect of the native VAM fungi on the growth of red clover in soil from the OBS-farms. One reason for this failure may have been the nutritional effect of the soil sterilization. This sterilization was carried out to obtain VAM free plants. The reinoculation of the sterile soil with a leaching of the unsterilized soil did not give the desired effect. Beside this, the high phosphorus status of the OBS soils makes it questionable whether a mycorrhizal growth response could be expected or not. The plants grew well on these soils and yielded at least twice as much as the red clover plants, grown under the same greenhouse conditions, in the effectivity test of the DOB-isolates (cf. table 4.6 and figure 4.1). It provides no evidence either whether or not a growth promoting effect of VAM does occur on the farms. In order to answer this question it is necessary to have more information about the root densities and the effect that higher root densities have on plant growth. As a matter of fact, the growth promoting abilities of VAM are visualized predominantly in cases in which enlargement of the root system (more soil is exploited for nutrients) responds with an increase in yield.

5 *THE INFLUENCE OF VARIOUS AGRICULTURAL PRACTICES ON VAM DEVELOPMENT*

5.1 INTRODUCTION

The mycorrhizal development as affected by various agricultural practices in long-term field experiments was studied on the Lovink-farm. These experiments were in view of their long-term character, assumed to be very valuable, especially in relation to the information that was available about their cropping history and the applied fertilizers. It was hoped that the results could help in explaining those found on the DOB/OBS-farms.

The VAM development was investigated mainly in winter wheat and potatoes and only in one experiment with oats.

5.2 MATERIALS AND METHODS

The experiments on the Lovink-farm which were involved in this study are described in chapter 3.
The methods and materials used for sampling and for the estimation of the VAM development and the inoculum potential are given in chapter 4. The number of samples taken for mycorrhizal analysis depended on the size of the plots and on the total number of samples that could be elaborated. Because of the observed variation in the first results, the number of samples was modified, but in most experiments three samples per plot were taken.

The yields of the crops were measured according to the normal procedures used on the Lovink-farm, and were kindly placed at my disposal.

5.3 RESULTS

5.3.1 *Nitrogen*

In most experiments on the Lovink-farm in which a particular factor is studied, also different amounts of nitrogen are applied. In this section the effects of nitrogen on VAM development in these various experiments are summarized.

The percentage of VAM in wheat decreased in most experiments with increasing amounts of nitrogen fertilizer (tables 5.1, 5.3, 5.4 and 5.7). There were exceptions, however, such as the results on the mini-DOB experiment (tables 5.11 and 5.12). As shown in the tables 5.3, 5.7, 5.9 and 5.10 the decrease of VAM development with increasing levels of nitrogen was not found in potatoes, indicating the influence of the host plant. The infection levels in the oat crop, grown in 1980 on the control plot of the green manure experiment (see 3.3), showed that the nitrogen effect was influenced by the previous crop. These results, given in fig. 5.1, show that the nitrogen effect was found after winter wheat but not after potatoes and sugarbeet.

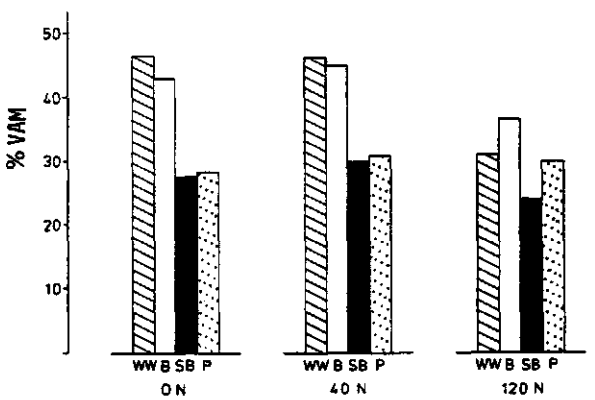

Figure 5.1 Influence of the previous crop on the VAM development in oats cv. Gambo at various levels of nitrogen given in the form of calcium nitrate in kg N ha^{-1} (Lovink-farm experiment 004, 1980).
P-fertilizer: 109 kg P_2O_5 ha^{-1} in the form of super phosphate 43%.
See for abbreviations page 6.

	Rotation I (WW, WW, WW, WW)									Rotation II (WW, SB, WW, WW, P, WW)					Rotation III (WW, P, WW, SB)					Rotation IV (O, P, WW, SB)		
field-plot	4-28	4-01	4-27	4-30	4-02	4-26	4-29	4-25	4-04	4-34	4-36	4-32	4-35	4-31	4-46	4-48	4-44	4-47	4-43	4-40	4-42	4-37
N-fertilizer[1]	0	0	30	60	60	90	120	150	150	0	60	90	120	150	0	60	90	120	150	0	60	150
1979																						
% VAM	55.3		45.3	37.6		29.7	25.2	24.9			47.0	34.8	35.5			30.9	20.0	16.8		oats		
s.d.	5.1		1.0	5.4		7.4	7.7	7.5			6.6	1.6	4.1			4.2	9.6	3.8		no assessment		
yield (kg ha^{-1})	2825		3934	4797		5144	4974	5167			4958	5382	5520			5781	6300	6243				
1980																						
% VAM	48.4	50.3		29.9	34.8			27.8	24.0	sugarbeet					potatoes					potatoes		
s.d.	6.5	5.4		6.4	9.0			8.5	3.5	no assessment					no assessment					no assessment		
yield (kg ha^{-1})	3550	3635		5036	5075			6631	6409													
1981																						
N-fertilizer[2]	0			90				225		0	90			225	0	90			225	0	90	225
% VAM	38.5			38.3				30.5		46.2	21.9			21.3	38.1	32.1			22.3	44.4	29.4	23.2
s.d.	12.7			6.3				12.2		3.3	8.1			2.5	9.3	2.1			4.7	16.5	8.2	0.7
yield (kg ha^{-1})	2346			5108				6549		3842	6306			8040	3469	5953			7698	3105	5831	7915

Table 5.1 VAM development in, and the yield of, winter wheat cv. Arminda grown in four different rotations and at various levels of nitrogen fertilizer application (Lovink-farm experiment 001). The % VAM is given as a mean of three replicates.
P-fertilizer: 108 kg P_2O_5 ha^{-1} given in the form of super phosphate 43%.

[1] N-fertilizer in the form of calcium nitrate in kg N ha^{-1} (1978, 1979, 1980).

[2] N-fertilizer in the form of ammonium nitrate in kg N ha^{-1} (1981).

5.3.2 *Crop rotation*

The results of the investigations on VAM development in the rotation experiment (001) are presented in table 5.1. Although the effect of the various rotations on VAM in winter wheat appears to be small, some trends can be discerned. The clearest effects were obtained in 1979, when rotation II seemed to stimulate the mycorrhizal development more than rotation I and III, notably at gifts of 60, 90 and 120 kg N ha^{-1}. Rotation III had the lowest level of VAM in the winter wheat after the nonmycorrhizal sugarbeet as a previous crop.

In 1980 winter wheat was grown only in the rotation with continuous wheat (rotation I). The presence of VAM differed not much from that of 1979.

In 1981 winter wheat was grown in all rotations. In this year the nitrogen was given in the form of calcium ammonium nitrate instead of in that of calcium nitrate. The differences in VAM development between the various nitrogen levels were smaller than in the previous years.

In the season of 1981 the inoculum potentials were estimated at fertilizer levels of 0 and 225 kg N ha^{-1}. The results show that there was a slight tendency towards a higher inoculum potential in rotation IV (table 5.2). Except for rotation IV, the number of infective diaspores per 100 g soil was higher in the rotations at the 0 kg N ha^{-1} level than at the 225 kg N ha^{-1} level. The subsequent level of mycorrhizal development was also higher at 0 kg N ha^{-1} than at 225 kg N ha^{-1}.

	Rotations							
	I		II		III		IV	
N-fertilizer[1]	0 N	225 N	0 N	225 N	0 N	225 N	0 N	225 N
% VAM	38.5	30.5	46.2	21.3	38.1	22.3	44.4	23.2
VAM inoculum potential[2]	377	336	399	279	387	220	633	633

Table 5.2 Comparison between the VAM inoculum potential in April 1981 and subsequent VAM development in winter wheat, cv. Arminda, at two nitrogen levels in the rotation experiment (001) at the Lovink-farm.

[1] N-fertilizer in the form of ammonium nitrate in kg N ha^{-1}.

[2] measured as number of VAM diaspores per 100 g soil.

5.3.3 *Green manure*

Green manure crops, mainly applied as undercrops and as second crops, are grown as a common agricultural practice in The Netherlands. This is not primarily done in view of nutrient availability for the following crops but for improvement of the soil structure and the enhancement of the organic matter content of the soil. The results that were obtained in 1979 (table 5.3) showed that rotation with red clover as a main crop for green manure had still a negative effect on VAM development even after three years. In the potato crop, this negative effect was found in all the examined nitrogen levels. In winter wheat, however, it is only found at the higher nitrogen applications (100 kg N ha^{-1} and 150/175 kg N ha^{-1}). The influence of the red clover crop on the yield of winter wheat was visible even three years after its cultivation (table 5.3). When yield and VAM development are compared, the latter seems to be primarily correlated with plant growth. However, in the rotation with red clover and at the highest nitrogen level the VAM development tended to be less than in the rotation without green manure, although similar yields were obtained. At 0 kg N ha^{-1}, the same development of VAM was found in both treatments, but the wheat yielded more in the red clover rotation.

In 1981 in the red clover rotation (III) the same difference as in 1979 was found only at the intermediate nitrogen level (60 kg N ha^{-1}) viz. a lower mycorrhizal infection (table 5.4). The expected nutritional effect on VAM at the 0 kg N ha^{-1} level was not found in the rotations I, II and III. Only with lucerne (IV) as previous crop, a slightly lower level of VAM was to be found. This corresponded with the better growth of the crop in rotation IV at 0 kg N ha^{-1}. The better VAM development in rotation II, grass-clover, (150 and 180 kg N ha^{-1}) corresponds partly with the reduced growth of the crop when it is compared with the other rotations.

The inoculum potentials were measured in soil collected in April 1981 and showed a tendency of being higher in the control rotation (oats in 1980) than in the rotation with the

winter wheat 1979						
	N-fertilizer[1]	0 N	50 N	100 N	150 N	175 N
no green manure (I)	% VAM ± s.d.	55.4 ± 7.2	42.1 ± 7.2	40.2 ± 5.7	28.9 ± 15.5	
	yield (kg ha^{-1})	2489	5285	6113	6527	
red clover (III)	% VAM ± s.d.	54.9 ± 5.4		26.9 ± 1.3		16.4 ± 4.8
	yield (kg ha^{-1})	3606		6589		6647
potatoes 1979						
	N-fertilizer[1]	0 N	100 N	140 N	220 N	300 N
no green manure (I)	% VAM ± s.d.	19.2 ± 5.1	27.7 ± 3.1		25.7 ± 6.8	24.9 ± 3.6
	yield (t ha^{-1})	30.1	48.7		55.7	60.1
red clover (III)	% VAM ± s.d.	11.7 ± 4.1		15.3 ± 1.8		17.5 ± 1.8
	yield (t ha^{-1})	32.5		52.3		57.9

Table 5.3 Effect of red clover as green manure on the VAM development in, and the yield of, winter wheat (cv. Arminda) and potatoes (cv. Bintje) in 1979 (Lovink-farm experiment 004).
The % VAM is given as a mean of six replicates. The yield is the mean yield of the sampled plots.
P-fertilizer: 108 kg P_2O_5 ha^{-1} in the form of super phosphate 43%.
[1] N-fertilizer in the form of calcium nitrate in kg N ha^{-1}.

winter wheat 1981								
	N-fertilizer[1]	0 N	60 N	85 N	150 N	155 N	180 N	225 N
rotation I	% VAM ± s.d.	46.4 ± 3.7		39.9 ± 10.2		22.4 ± 6.9		20.9 ± 6.0
oats in 1980	yield (kg ha^{-1})	2504		5921		7419		7847
rotation II	% VAM ± s.d.	44.6 ± 2.4	41.9 ± 4.9		30.7 ± 7.8		30.6 ± 8.1	
grass-clover ley in 1980	yield (kg ha^{-1})	3051	5505		6933		7201	
rotation III	% VAM ± s.d.	46.5 ± 3.9	22.9 ± 3.2		22.8 ± 2.5		24.0 ± 11.8	
red clover in 1980	yield (kg ha^{-1})	4281	6210		7449		7358	
rotation IV	% VAM ± s.d.	39.8 ± 6.6			20.3 ± 5.9			
lucerne in 1980	yield (kg ha^{-1})	5528			7321			

Table 5.4 Effect of different green manure crops in the VAM development in, and the yield of, winter wheat cv. Arminda (Lovink-farm experiment 004). The winter wheat was grown in 1981, the year after the growth of the green manure crops. The % VAM is given as a mean of three replicates.

P-fertilizer: 108 kg P_2O_5 ha^{-1} in the form of super phosphate 43%.

[1] N-fertilizer in the form of ammoniumnitrate in kg N ha^{-1}.

	Green manure applications					
	I		II		III	
N-fertilizer[1]	0 N	225 N	0 N	180 N	0 N	180 N
% VAM	46.4	20.9	44.6	30.6	46.5	24.0
VAM inoculum potential[2]	802	633	423	243	340	191

Table 5.5 Comparison between the VAM inoculum potential in April 1981 and the subsequent VAM development in winter wheat, cv. Arminda, at two nitrogen levels in the green manure experiment (004) at the Lovink-farm.

[1] N-fertilizer in the form of ammonium nitrate in kg N ha^{-1}.

[2] measured as number of VAM diaspores per 100 g soil.

green manure crops (table 5.5). They were in general higher at the 0 kg N ha^{-1} level than at the 180 kg N ha^{-1} level. High inoculum potentials, however, did not always correspond with high infection levels (compare rotation I, 225 kg N ha^{-1} with rotation II, 180 kg N ha^{-1} in table 5.5).

5.3.4 *Soil tillage*

The effect of different soil tillage practices on VAM in winter wheat is shown in table 5.6. In 1979 when only three samples per plot were examined it was noted that the plot with the greatest compaction of the soil (minimal tillage) had the highest frequency of VAM. It was lowest in the plot with traditional tillage. In 1980 similar results were obtained.

	field	1979 % VAM ± s.d.	yield (kg ha^{-1})	1980 % VAM ± s.d.	yield (kg ha^{-1})
minimal tillage	29	32.5 ± 9.6	6596	22.6 ± 7.2[a]	7817
intermediate tillage	26	25.8 ± 6.4	6620	15.4 ± 5.6[b]	7567
traditional tillage	36	18.7 ± 7.6	6364	8.3 ± 1.5[c]	7051

Table 5.6 Effect of soil tillage on VAM development in, and the yield of, winter wheat, cv. Arminda (soil tillage experiment 007, at the Lovink-farm).
P-fertilizer: 108 kg P_2O_5 ha^{-1} given in the form of super phosphate 43%.
N-fertilizer: in 1979 93 kg N ha^{-1} in the form of calcium nitrate; in 1980 117 kg ha^{-1} in the form of ammonium nitrate. The % VAM is a mean of three replicates in 1979 and of eight replicates in 1980.
The values with the same letter are not significantly different ($p<0.05$; t-test).

potatoes 1979							
		N-fertilizer	0 N	120 N	150 N	200 N	250 N
- FYM	+ 107 kg P_2O_5 ha^{-1}	% VAM ± s.d.	6.5 ± 0.4		6.8 ± 2.0		8.5 ± 2.4
	+ 270 kg K_2O ha^{-1}	yield (t ha^{-1})	31.2		49.0		49.8
+ FYM	+ 47 kg P_2O_5 ha^{-1}	% VAM ± s.d.	5.4 ± 1.5	4.4 ± 1.6		5.5 ± 1.1	
	+ 107 kg K_2O ha^{-1}	yield (t ha^{-1})	38.1	49.3		53.6	
winter wheat 1980							
		N-fertilizer	0 N		50 N		125 N
- FYM	+ 108 kg P_2O_5 ha^{-1}	% VAM ± s.d.	44.4 ± 4.5		29.6 ± 1.9		19.0 ± 7.0
		yield (kg ha^{-1})	5268		5860		6808
+ FYM	+ 108 kg P_2O_5 ha^{-1}	% VAM ± s.d.	37.8 ± 9.1		29.0 ± 11.7		14.5 ± 6.8
		yield (kg ha^{-1})	5310		6147		6488

Table 5.7 Effect of farmyard manure (FYM) on the VAM development in, and the yield of, potatoes cv. Bintje in 1979 and winter wheat cv. Arminda in 1980 in the farmyard experiment 010 at the Lovink-farm. The last FYM application was in the autumn of 1978.
The phosphorus and potassium in the FYM is compensated for in the control treatment (-FYM) by additional P and K fertilizers in 1979.
The N-fertilizer was given in the form of calcium nitrate in kg N ha^{-1}. The % VAM is a mean of six replicates in potatoes and three replicates in winter wheat.

5.3.5 *Farmyard manure*

In the farmyard manure experiment (010) root samples of potatoes and winter wheat were examined in 1979 and 1980 respectively. In the autumn of 1978 the last farmyard manure (FYM) application was carried out. As shown in table 5.7 FYM had little influence on mycorrhizal development in the potato crop in this experiment. No effect of the nitrogen fertilizers was found. In the roots of winter wheat the VAM development showed a tendency to be somewhat lower in the FYM treatment.

5.3.6 *Phosphorus*

In the phosphorus experiment (012) five plots were examined that had, for Dutch circumstances, extremely high and low levels of available P. As indicated in table 5.8 the water soluble phosphorus (P_w-value) was measured in February 1978 and resulted in low and high P_w-values. A month later some of the plots were fertilized with super phosphate at a rate of 500 kg P_2O_5 ha^{-1}. After the following potato crop the P_w-values were measured again. On the basis of these figures a choice was made of the plots to be examined in 1979 for mycorrhizal infection in wheat. The highest level was found in the plot with the lowest amount of available P. In the other plots the wheat roots were mycorrhizal for about 40%. The yield of the crop in the plot with a low P level was lower than in the other plots.

plot number	12	5	30	38	41
P_w-value, 2/1978	5	32	4	39	37
kg P_2O_5 ha^{-1}, 3/1978	0	500	500	0	500
P_w-value, 10/1978	7	92	30	22	68
% VAM, 1979	56.7	41.5	37.7	38.7	37.6
s.d.	1.9	4.3	5.3	6.4	2.3
P_w-value, 10/1979	5	46	17	15	41
grain yield (kg ha^{-1})	5645	6206	6064	5947	5932
straw yield (kg ha^{-1})	5626	6270	5982	5867	5964

Table 5.8 Effect of the phosphorus level in the soil on the VAM development in, and the yield of, winter wheat cv. Durin in 1979 (Lovink-farm experiment 012). The % VAM is a mean of three replicates. N-fertilizer: 77.5 kg ha^{-1} as calcium nitrate. P_w is given in mg P_2O_5 per litre soil. P-fertilizer in the form of super phosphate 43%.

potatoes 1979

	object I			object II			object III		
field-plot	14-58	14-56	14-55	14-70	14-68	14-67	11-46	11-44	11-43
N-fertilizer in 1978	77.5	77.5	77.5	62.0	62.0	62.0	autumn 1978 35 t FYM ha^{-1}		
crop in 1978	spring barley			spring barley + Italian rye-grass as undercrop			ley		
N-fertilizer in 1979	0	180	300	0	180	300	0	150	250
% VAM	17.5	11.0	10.9	15.1	12.2	12.2	17.2	12.4	21.4
s.d.	0.8	2.4	2.6	1.5	0.2	2.5	1.5	4.4	1.3
yield (t ha^{-1})	32.0	51.9	55.5	42.4	57.3	60.3	46.7	60.2	65.3

Table 5.9 VAM development in, and the yield of, potatoes cv. Bintje grown in the mini-DOB experiment at the Lovink-farm (006) at various levels of nitrogen (1979). The objects I, II and III are comparable with, respectively, the KA, KL and WW-farm on the previous DOB-farms. Nitrogen was given in the form of calcium nitrate in kg N ha^{-1}.
P-fertilizer: 108 kg P_2O_5 ha^{-1} given in the form of super phosphate 43%.
K-fertilizer: 270 kg K_2O ha^{-1}. The % VAM is a mean of two replicates.

potatoes 1980

	object I			object II			object III		
field-plot	15-52	15-50	15-49	15-64	15-62	15-61	16-40	16-38	16-37
N-fertilizer in 1979	75.5	75.5	75.5	62	62	62	autumn 1979 27 t FYM ha^{-1}		
crop in 1979	spring barley			spring barley + Italian rye-grass & clover as undercrop			ley		
N-fertilizer in 1980	0	180	300	0	180	300	0	150	250
% VAM	8.0	7.0	7.1	9.8	6.0	6.4	10.2	9.5	9.4
s.d.	1.0	1.9	3.4	3.2	2.4	3.5	6.3	3.4	2.5
yield (t ha^{-1})	33.0	45.8	45.9	41.5	56.9	61.1	46.1	60.9	65.5

Table 5.10 VAM development in, and the yield of, potatoes cv. Bintje grown in the mini-DOB experiment (006) at the Lovink-farm at various levels of nitrogen (1980). The objects I, II and III are comparable with, respectively, the KA, KL and WW-farm on the previous DOB-farms. Nitrogen was given in the form of calcium nitrate in kg N ha^{-1}.
P-fertilizer: 108 kg P_2O_5 ha^{-1} given in the form of super phosphate 43%.
K-fertilizer: 270 K_2O ha^{-1}. The % VAM is a mean of three replicates.

winter wheat 1980

	object I			object II			object III		
field-plot	14-52	14-50	14-49	14-64	14-62	14-61	11-40	11-38	11-37
N-fertilizer in 1979	0	180	300	140	140	140	93	93	93
crop in 1979	potatoes			potatoes			potatoes		
N-fertilizer in 1980									
- basic manure	62	62	62	47	47	47	31	31	31
- top dressing	0	60	100	0	60	100	0	60	100
% VAM	19.5	23.9	25.9	25.6	22.8	30.8	28.5	34.6	40.2
s.d.	5.4	8.1	7.6	3.4	4.1	6.4	8.3	8.0	4.0
yield (kg ha^{-1})	6286	7145	7569	6935	7507	7449	6965	7885	7838

Table 5.11 VAM development in, and the yield of, winter wheat cv. Arminda grown in the mini-DOB experiment (006) at the Lovink-farm at various levels of nitrogen (1980). The objects I, II and III are comparable with, respectively, the KA, KL and WW-farm on the previous DOB-farms. Nitrogen was given in the form of calcium nitrate.
P-fertilizer: 108 kg P_2O_5 ha^{-1} in the form of super phosphate 43%.
The % VAM is a mean of three replicates.

winter wheat 1981	object I			object II			object III		
field-plot	15-58	15-56	15-55	15-70	15-68	15-67	16-46	16-44	16-43
N-fertilizer in 1980	0	180	300	140	140	140	93	93	93
crop in 1980	potatoes			potatoes			potatoes		
N-fertilizer in 1981									
- basic manure	78	78	78	59	59	59	39	39	39
- top dressing	0	75	125	0	75	125	0	75	125
VAM inoculum potential	175	-	344	460	-	670	599	-	344
% VAM	26.5	26.9	28.2	34.7	34.2	22.0	42.0	32.6	25.3
s.d.	11.9	11.1	1.2	12.6	8.7	4.4	6.3	7.5	10.6
yield (kg ha^{-1})	6150	7523	7924	6118	7272	7898	6537	7524	7491

Table 5.12 VAM development in, and the yield of, winter wheat cv. Arminda grown in the mini-DOB experiment (006) at the Lovink-farm at various levels of nitrogen (1981).

The VAM inoculum potential was based on the number of VAM diaspores in 100 g soil sampled in April 1981. The objects I, II and III are comparable with, respectively, the KA, KL and WW-farm on the previous DOB-farms. Nitrogen was given in the form of ammonium nitrate; in the previous crop as calcium nitrate in kg N ha^{-1}.

P-fertilizer: 108 kg P_2O_5 ha^{-1} in the form of super phosphate 43%.

The % VAM is a mean of three replicates.

5.3.7 *Farming systems*

In the mini-DOB experiment (006) the VAM development was examined in potatoes and winter wheat. This was done twice during two successive years in the same field. In the used rotation potatoes are followed by winter wheat. This implies that the winter wheat was sampled on the same field as the potatoes in the previous year. In table 5.9 and 5.10 the percentage VAM is given together with additional information on the fertilizer supply of the potatoes and of the previous crop. In 1979 the VAM development was somewhat higher than in 1980. In the latter year the VAM infection levels showed less variation viz. that the application of different amounts of N-fertilizer has little influence on the VAM development in potatoes (see 5.3.1). There was a slight trend towards a little more VAM in potatoes grown in object III.

The mycorrhizal development in winter wheat differed in 1980 from that in 1981 (table 5.11 and 5.12). In 1980 the levels of mycorrhizal infection increased with decreasing amounts of N-fertilizer applied as basic manure. At the highest levels of top dressing, however, the largest VAM development was found. This is opposite to what was expected. In 1981 the inoculum potential was therefore measured in April as well as the subsequent mycorrhizal formation in June. In 1981 the VAM development in winter wheat showed however, not the same pattern as in 1980. In this case the usual pattern was obtained viz. increasing VAM levels with decreasing amounts of nitrogen. The crop responded remarkably to the top dressing by higher yields; in 1981 more than in 1980. The inoculum potential that was measured was variable and showed neither a clear correlation with the mycorrhizal infection nor with the amount of nitrogen fertilizer.

5.3.8 *Summary*

The observed effects of the different agricultural practices on the VAM development can be summarized as follows:

- The effect of nitrogen on the mycorrhizal symbiosis is host dependent. In winter wheat increased amounts of nitrogen fertilizer gave a decreased VAM development. In potatoes no such effect was found.
- The above mentioned N-effect can be influenced by the previous crop.
- The previous crop may affect the mycorrhizal condition of its successor via its effect on the inoculum potential. Not in every experiment however could the inoculum potential account for the observed differences.
- The effect of green manure crops on VAM development was variable. It depended on the green manure crop that was used.
- Soil tillage influenced markedly the mycorrhizal development in winter wheat. Tillage practices that lead to compaction of soils enhanced the development of VAM in this crop.
- Farmyard manure had a slight negative effect on mycorrhiza formation.

5.4 DISCUSSION

5.4.1 *Nitrogen*

There are several reports on the effect of nitrogen on the mycorrhizal symbiosis. The ones dealing with agricultural crops are mostly field observations concerning the recovery of spores or the assessment of mycorrhizal development.

In a wheat field Hayman (1970) found a lower number of spores after application of nitrogen fertilizer. This decrease in spore numbers was correlated with a decrease of VAM infection. He even suggested that the nitrogen effect was stronger than the phosphorus effect. In a barley crop a similar effect of nitrogen on VAM development was found by Jensen & Jakobsen (1980) in different soils. This was res-

tricted, however, to treatments in which P-fertilizer was applied. The results of Kruckelmann (1973, 1975) were more variable. In one soil (silty clay loam from Rothamsted, U.K.) on which wheat was grown continuously, he found a decrease in the number of spores after various fertilizer applications with increasing amounts of nutrients. In another soil (loamy sand from Braunschweig, G.F.R.) on which oats were grown after rye after potatoes similar amounts of fertilizer caused an increase in spore numbers. In soil from a sugarbeet field on which oats had grown as a previous crop he did not find any effect of different amounts of N-fertilizer applied during eleven years.

The reports on the effect of nitrogen on VAM in permanent grassland are not unanimous either. Sparling & Tinker (1978) found no effect, but Mejstrik (1977) found fewer mycorrhizas in meadows with larger amounts of nitrogen in the soil.

The results of our own investigations are in accordance with earlier observations that nitrogen decreases VAM development in wheat. In potatoes, however, no effect of nitrogen on VAM development was found. There was in one instance even a tendency of a stimulating effect of N-fertilizer on mycorrhizal development (table 5.3). In oats the nitrogen effect was influenced by the previous crop (fig. 5.1).

It is difficult to give an explanation for the effect of nitrogen on VAM development and its modification by the species of host plant. It could be based on factors acting in and/or outside the roots. Chambers et al. (1980) showed an effect of the kind of fertilizers used. Not only did NH_4^+-ions reduce VAM development more than NO_3^--ions, but also Na_2SO_4 application decreased VAM. They concluded on the basis of a lower number of entry points formed by the mycorrhizal fungus in the roots of *Trifolium subterraneum* that the salts had a negative effect on the VAM fungus in the pre-infection phase. They also found a reduction of the VAM development. Such a salt effect on the pre-infection phase gives no explanation for the absence of a nitrogen effect in potatoes.

The influence of the previous crop on the nitrogen effect in an oat crop suggests that the inoculum potential is also

involved. Nutritional after-effects are not to be expected, because the plots received comparable amounts of nitrogen during many years.

The absence of a nitrogen effect in potato is probably based on a different distribution of assimilates in the plant in the case of nitrogen deficiency. The tubers act as a sink for the assimilates so that no increased level of carbohydrates occurs in the roots. In various crops carbohydrate levels are reported to be positively correlated with levels of VAM infection (Azcon & Ocampo, 1981; Daft & El-Giahmi, 1978).

5.4.2 *Crop rotation*

In attempts to understand the effects of various rotations on VAM development it is necessary to discuss the single factors that determine the rotational effects.

- Plant nutrition

Because the crops used in the rotations are usualy fertilized differently, after-effects based on fertilizer residues can be expected.

Crop residues can also influence the levels of available plant nutrients. The amount of crop residue is dependent on the agricultural practice, e.g. whether the cereal straw is ploughed in or not.

- Inoculum potential

The importance of this factor is illustrated by a number of investigators. Kruckelmann (1973) showed that the numbers of spores produced in field crops grown in continuous culture of 16 years can vary largely between crop species. In different rotations the numbers of spores from fields with the same crop were dependent on the cropping history. The increase and decrease of spore numbers is also shown by T.F. Smith (1980) in three rotations with pastures and wheat. He found a decrease of spore numbers during a wheat crop and an increase during a pasture period: A similar increase in inoculum potential was found during the ley periods on the DOB-farms (chapter 4).

In P-deficient soils, the effect of the initial level of inoculum, can decrease during the vegetation period (Ocampo, 1980, using pot experiments). Schenck & Kinloch (1980) reported on a seven year's investigation of continuous growing of various crops on a cleared woodland site. After the first three years they found a decline in the VAM development in cotton, sorghum and bahia grass. This decline had almost disappeared a few years later. In their experiment the VAM development was highly correlated with the number of spores. They considered the decline in agreement with the results of Strzemska (1975), but this was true only with few of the reported data. The correlation between VAM and yield which can also be found in the results of Strzemska explains probably better the fluctuations in number of VAM fungal spores and VAM development than the growth of the crops in continuous culture only, as Schenck & Kinloch (1980) supposed. However, Schenck & Kinloch reported no yields, therefore their decline cannot be shown to be attributed to a better growth of the crops.

- Inhibition of VAM development by previous crops.

Hayman et al. (1975) suggested that this might occur in crop rotations with non-host species. This was based on their findings that mixed growing of onions and swedes resulted in far less VAM in onions than when they were grown alone. They concluded that exudates of the swedes inhibited VAM formation in onions as well. Ocampo & Hayman (1981) studied this in further detail in pot experiments. Their results show no inhibitory effect of sugarbeet, oil seed rape and cabbage as previous crops. On the contrary, the early establishment of VAM in barley, lettuce and maize was stimulated when the roots of the previous crop were retained in the soil.

The above mentioned factors made it difficult to relate the mycorrhizal development directly with the used rotations. In figure 5.2 the percentage VAM has therefore been related with the wheat yield. From this it can be seen that at any particular yield the percentage VAM was larger in rotation II than in rotation I and III.

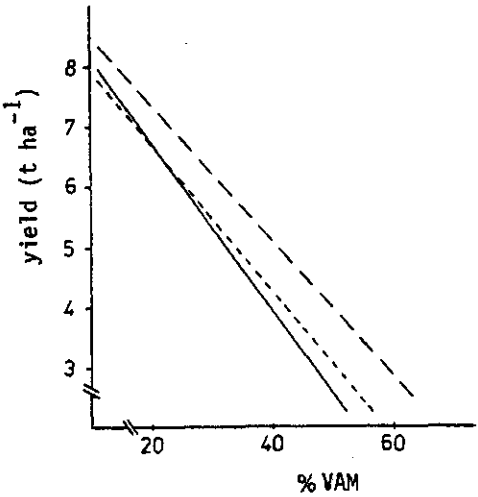

Figure 5.2 Relation between VAM development and yield of winter wheat in the rotation experiment on the Lovink-farm (experiment 001; rotations I, II and III). The relations are expressed by linear regression lines based on data obtained during the period 1979-1981.
I (———): y=9.3 - 0.14x (r= -0.76; n=15), II (----): y=9.5 - 0.11x (r= -0.88; n=6), III (.....): y=9.2 - 0.12x (r= -0.74; n=6).

5.4.3 *Green manure*

The effect of green manure on VAM development is comparable with that of crop rotation by its influence on the nutritional status of the soil and the inoculum potential of the VAM fungi. The obtained results show only small effects of green manure applications. These applications influenced VAM development in winter wheat only at intermediate and high nitrogen levels, but had no effect in the treatments without additional nitrogen. The latter might be due to the fact that a maximum level of VAM seems to be reached at low nutrient availabilities.

The lower level of VAM infection in potatoes, three years after the cultivation of red clover, cannot be explained by nutritional effects. Phosphorus and potassium were adequately available, and the nitrogen applications had no clear influence on the presence of mycorrhizas. The only reasonable explanation seems a lower VAM inoculum potential. The latter was, however, not estimated.

The results indicated that green manure did have some influence on VAM. This effect can not yet be explained satisfactorily.

5.4.4 *Soil tillage*

The results obtained in the soil tillage experiment are partly in agreement with the results of Kruckelmann (1973). He found a lower number of spores in the treatment with the largest disturbance of the soil. In a comparable situation on the Lovink-farm the lowest VAM level was obtained. In the minimal tillage treatment the highest level was found. This might be due to the fact that in the latter situation lower amounts of nutrients are available. This was e.g. shown by Bakermans & de Wit (1970) who found that on compacted soils an extra 20-40 kg N ha^{-1} was needed to obtain the same maximum yield of small grains as on traditionally cultivated soils. This could suggest that the wheat crop in the minimal tillage treatment had a N-deficiency, but the crop on the compacted soil gave higher yields (table 5.6). There was probably no N-deficiency in the minimal tillage treatment, while on the other hand there may have been a supra optimal nitrogen level in the traditional tillage treatment. The latter can also result in a lower VAM development as will be described in chapter 6.

5.4.5 *Farmyard manure*

In the farmyard manure experiment on the Lovink-farm the application of FYM had a decreasing effect on the VAM development in winter wheat and potatoes. The effect was small, but so was the influence on the growth of the crops (table 5.7). Farmyard manure can give remarkable effects on mycorrhizal development, both positive and negative (Kruckelmann, 1973; Jensen & Jakobsen, 1980). FYM and more in general organic substrates, can influence the availability of plant nutrients in opposite ways. An important parameter in this respect is the C:N ratio of the substrate. In the case of a high C:N ratio the soil micro-organisms will assimilate the

carbon and use a part of the nutrients in addition. This results in a stimulating effect on the VAM development. In the case of a low C:N ratio the opposite will occur. The type of the carbon source is also of importance (Nyabyenda, 1977; Peuss, 1958). Phosphorus availability which influences the activity of soil micro-organisms may also be involved (Tesarova, 1972). Another factor may be the type of organisms which use the substrate.
Organic substrates may also affect the pH and structure of the soil. These are well known to influence the mycorrhizal symbiosis. Decomposition products may also have inhibitory effects.

Thus the influence of FYM on VAM can vary greatly and depends on the particular processes which are affected.

5.4.6 *Phosphorus*

Many papers present evidence for large effects of P-fertilizers on aspects of the mycorrhizal symbiosis (Daft & Nicolson, 1966; Mosse, 1973; Sanders, 1975 and others).

Our own results show high levels of VAM, even in the treatments with large amounts of available phosphorus. As will be described in 6.3.1, the level of available nitrogen influences the effect of P-fertilizer on VAM development. The nitrogen level may account for the small differences between the various phosphorus levels. This seems in agreement with the results of Hayman (1975) that the effects of P-fertilizer were not as marked as the effects of nitrogen fertilizer in field experiments.

5.4.7 *Farming systems*

The different farming systems in the mini-DOB experiment had little effect on mycorrhizal development. When the yield is related to the percentage VAM, the VAM level in object III tended to be more at comparable yields (figure 5.3). This indicates probably a slightly stimulating effect of the application of pastures in the rotation. As shown in chapter 4 and by T.F. Smith (1980), pastures can build up higher inoculum potentials.

5.4.8 *Concluding remarks*

The results obtained lead to the general conclusion that the agricultural practices studied, influence the development of VAM mainly by differences in nutrient availability and presumably also by differences in VAM inoculum potential.

The results from the experiments on the Lovink-farm provide evidence that the differences in VAM development on the farms of the OBS-project are mainly based on nitrogen deficiency. The expected increase in VAM inoculum potential and the decrease of the phosphorus availability on the BD-farm will undoubtedly lead to a better development of VAM in potatoes in the coming years.

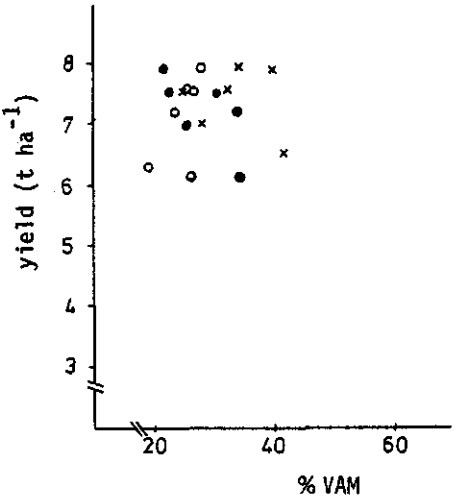

Figure 5.3 Relation between VAM development and yield of winter wheat in the mini-DOB experiment (006) on the Lovink-farm. Data represent observations on VAM done in 1980 and 1981. (o): object I (KA-farm); (•): object II (KL); (x): object III (WW).

6 *GREENHOUSE STUDIES OF FACTORS WHICH AFFECT VAM DEVELOPMENT*

6.1 INTRODUCTION

In this chapter experiments will be described that were performed in the greenhouse in order to arrive at a better understanding of the field situations. These experiments were carried out with wheat.

In arable soils in The Netherlands phosphorus levels are in most cases high and the usual application of nitrogen fertilizers is large. Yet VAM is present under these circumstances. It was therefore decided to study the interaction between phosphorus (P) and nitrogen (N) in relation to the development of VAM at various levels of P and N. This interaction received thus far little attention in mycorrhizal research. Experiment 1 deals with this aspect. The effect of nitrogen in this experiment appeared to be less pronounced than in the field. It was suspected that the responsible factor might be the inoculum potential of the VAM fungi. For that reason a second experiment deals with that factor (experiment 2).

In the field plants are grown together as a crop and not as individuals as in pot experiments. The factor of plant density will have an important effect on many parameters in the root zone and thereby on VAM. In a third experiment the factor plant density was therefore examined.

6.2 MATERIALS AND METHODS

6.2.1 *Soils*

Use was made of two soils, A and B, consisting of light calcareous clay loam, with low nutrient levels. These soils were autoclaved at 121 °C during 1½ hour. Soil A was used in experiment 1. Soil B was used for the experiments 2 and 3. It consisted of a new bulk of soil, with even lower amounts

Soil A:

milli-equivalents per litre soil					
NH_4^+	0.2	NO_3^-	0.8	water soluble P	1.2 mg per litre soil
K^+	1.0	Cl^-	0.8	conductivity	2.1 mS cm^{-1} (25 °C)
Na^+	5.6	SO_4^{2-}	48.0	pH-KCl	7.0
Ca^{2+}	46.4	HCO_3^-	1.2	organic matter	2.3%
Mg^{2+}	7.6			$CaCO_3$	6.1%

Soil B:

milli-equivalents per litre soil					
NH_4^+	0.2	NO_3^-	0.2	water soluble P	0.8 mg per litre soil
K^+	0.8	Cl^-	0.6	conductivity	0.3 mS cm^{-1} (25 °C)
Na^+	0.6	SO_4^{2-}	3.4	pH-KCl	7.1
Ca^{2+}	3.4	HCO_3^-	2.4	organic matter	2.0%
Mg^{2+}	0.8			$CaCO_3$	4.2%
				P-Al	11.0

Table 6.1 Data of the soils used in greenhouse experiments. These soils came from a location in the Flevo Polder near Lelystad. Soil A was used in experiment 1 as described in 6.2.1. Soil B was used in the other greenhouse experiments.

of extractable N and P. Data concerning soil A and B are presented in table 6.1.
In experiment 1 and 2 the pots contained 1.5 kg of soil (on a dry weight basis); in experiment 3 the cylinders that were used (20 cm in diameter, 40 cm high) were filled with 13.3 kg of soil (on a dry weight basis). During the three experiments the water content of the soil was between 45 and 65% of the upper plasticity limit. The soil in the pots was covered with a layer of fine gravel in order to reduce evaporation from the soil and to maintain a constant and uniform humidity in the pots.

6.2.2 *Application of fertilizers*

- Experiment 1. The soil used in this experiment contained 21 mg of available nitrogen per pot. By applying various amounts of calcium nitrate the level of total available nitrogen was brought to 21, 43, 65, 87, 121, 155, 221 and 421 mg per pot. Half of the additional nitrogen was given eight days after transplanting of the germinated seeds. The other half was given when 50% of the available nitrogen at

the 221 mg level had been assimilated. This moment was calculated on the basis of the amount of water which had been evaporated and on the assumption that 200 ml of evaporated water is equivalent to 1 g of dry matter or 30 mg of nitrogen. At the start of this experiment three different levels of phosphorus were established at the nitrogen levels of 43, 87 and 221 mg N per pot. To this end calcium biphosphate ($Ca(H_2PO_4)_2 \cdot H_2O$) was given in amounts of 0, 6.2 and 49.8 mg P per pot. All the treatments received 148 mg K per pot of magnesium sulphate. Under the conditions of this experiment the pots with a N level of 221 mg and given 49.8 mg P were assumed to have an optimum nutrient level.

- Experiment 2. In this experiment two nitrogen levels were established, namely with 52.4 mg N per pot (N-1) and with 408.4 mg per pot (N-2). The additional nitrogen was given at intervals in the form of calcium nitrate. Two phosphate levels were also established, namely one with no additional phosphate (P-1) and one with an additional amount of 24.9 mg P per pot given in the form of calcium biphosphate (P-2). The amounts of potassium and magnesium were 14 mg K per pot and 85 mg Mg per pot.

- Experiment 3. In this experiment two P-levels were established. Level P-1 received no additional phosphate; level P-2 received 1402 mg P per pot in the form of potassium phosphate. The latter was equivalent to 522 kg P_2O_5 ha^{-1}. Additional nitrogen was given as ammonium nitrate to a total amount of 42 mg N per plant.

6.2.3 *Inoculation with Glomus mosseae*

In the three experiments different methods of inoculation with *G. mosseae* were used. In experiment 1 the inoculum of *G. mosseae* consisted of 0.8 g of freshly washed tomato roots, which were infected with the fungus. This inoculum was spread in two layers, namely one at 3 cm from the bottom of the pot and the other one at 4 cm from the surface of the soil.

In experiment 2 the effect of the method of inoculation itself was studied. The method (I) used in experiment 1 was compared with one in which soil containing inoculum was mixed through the soil in the pot (method II). This inoculum-soil mixture was derived from pot cultures with mycorrhizal tomato plants (*G. mosseae*) and consisted of a mixture of soil with small root fragments and spores. The ratio between sterilized soil and inoculum-soil was 10:1.

In experiment 3 the same method of inoculation with inoculum-soil was used as in experiment 2. In the control treatments autoclaved roots or autoclaved inoculum-soil were used.

6.2.4 *Host plant*

In all three experiments spring wheat cv. Bastion, was used as host plant. The seeds were disinfested in 2% NaClO for two minutes and were subsequently allowed to germinate in sterilized sand. The germinated seeds with hypocotyls of 0.5-1.0 cm were planted in the pots: four plants per pot in experiment 1, five plants per pot in experiment 2 and in experiment 3 two or twelve plants per pot.
In experiment 3 the number of twelve plants per pot was comparable to plant densities as used in the field.

6.2.5 *Growing conditions*

The experiments were carried out under favourable natural light conditions (March-September) in the greenhouse. The pots were placed in randomized position and moved a little every two or three days in order to avoid location effects. The temperature varied between 22 and 28 °C.

6.2.6 *Measurements*

Dry weight and the percentage VAM was recorded in all the experiments. In experiment 3 the total length was estimated by means of the line-intersection method (Ambler & Young, 1977). The root samples used for the total root length measurements, were obtained by use of a Folsom plankton sample

splitter (McEwen et al., 1954). Each root sample was suspended in water and was divided in equal aliquots until a subsample of about 0.25 g root pieces (fresh weight) was obtained. These subsamples were used for further measurements. Three replicates were made of each sample.

6.3 RESULTS

6.3.1 *Experiment 1: The interaction between levels of N and P in the soil in relation to VAM development in spring wheat, and the growth of the plants*

- The effect on the mycorrhizal development.

The results of this experiment are given in table 6.2 and fig. 6.1. At the lowest phosphorus level, an initial increase of VAM frequency with increasing nitrogen levels was observed, followed by a decrease at the highest nitrogen levels. This increase and subsequent decrease was almost parallelled by the effect of nitrogen application on plant growth. The availability of nitrogen was a growth limiting factor at this low

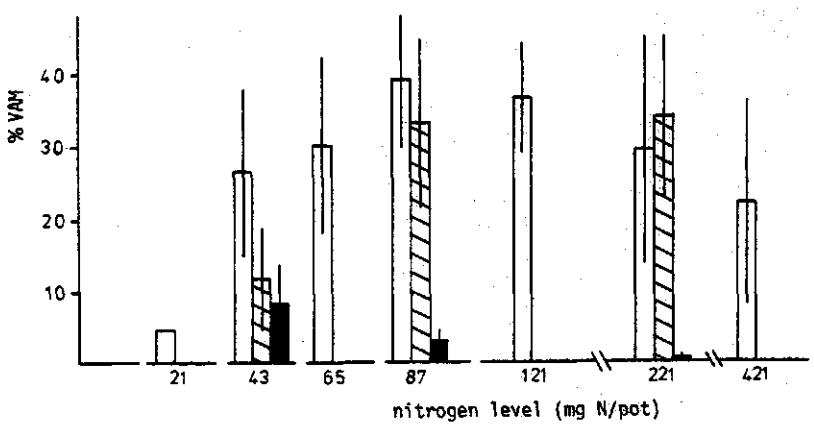

Figure 6.1 Development of Glomus mosseae in spring wheat cv. Bastion as affected by various amounts of nitrogen and phosphorus fertilizer.
P-fertilizer: □ 0 mg P per pot; ▨ 6.2 mg P per pot; ■ 49.8 mg P per pot.

P-fertilizer (mg P per pot)		0	0	6.2	49.8	0	0	6.2	49.8	0	0	0	6.2	49.8	0
N-level	(mg N per pot)	21	43	43	43	65	87	87	87	121	155	221	221	221	421
+ VAM	dry weight shoots (g per pot)	1.79	2.52	2.58	2.84	3.11	3.38	3.84	3.99	3.53	3.52	3.20	4.13	5.14	2.68
	s.d.	0.10	0.09	0.16	0.14	0.30	0.35	0.28	0.24	0.45	0.23	0.50	0.14	0.38	0.14
	% VAM	4.5	26.0	11.7	8.0	29.7	38.8	32.9	2.7	36.6	-	28.8	34.0	0.4	22.0
	n	1	4	4	5	5	3	3	5	4	0	3	4	3	4
	s.d.	-	11.6	6.9	5.5	12.1	9.3	11.6	1.4	7.5	-	15.6	11.3	0.5	13.6
- VAM	dry weight shoots (g per pot)	1.40	2.16	2.40	2.47	2.57	3.22	3.65	3.84	3.37	3.10	2.82	3.72	4.96	2.72
	s.d.	0.14	0.28	0.30	0.17	0.22	0.39	0.13	0.42	0.14	0.46	0.49	0.42	0.40	0.47
	% VAM	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	n	3	4	4	5	4	5	4	5	4	3	4	2	3	4

Table 6.2 The influence of N- and P-fertilizer on the development of VAM in spring wheat, cv. Bastion, and the dry weight of the shoots. The dry weights of the shoots are means of five replicates. The plants were harvested one week after the beginning of the flowering of the plants.

phosphorus level until 121 mg N per pot was available. At these higher levels of nitrogen, the growth of the plants began to decrease too, probably due to a combination of P-deficiency and overmanuring with nitrogen. A number of root samples was lost by accident; therefore the average percentage of VAM infection was based on different numbers of samples. This loss was felt stronger because of the considerable variation in the VAM frequency.

At the intermediate phosphorus level (6.2 mg P per pot) the average VAM frequency rose from 12 to 33% when the available amount of nitrogen was increased from 43 mg per pot to 87 mg per pot. At the lower nitrogen levels, nitrogen was the growth limiting factor because more nitrogen gave more plant growth. The N-deficiency was small at 87 mg N per pot and at this nitrogen level there was no clear P-deficiency. Phosphorus became growth limiting at a nitrogen level of 221 mg per pot.

At the highest phosphorus level (49.8 mg P per pot) the mycorrhizal development decreased with increasing amounts of nitrogen. Nitrogen was growth limiting at the 43 and 87 mg N level. In this situation the percentage VAM was negatively correlated with the growth of the plants.

- The effect of VAM on plant growth.

The mean dry weight of the shoots was higher in most treatments after inoculation with *G. mosseae*. The difference was, however, not significant in many of the treatments. It is moreover questionable whether the increased growth can be explained by mycorrhizal symbiosis. For example, in the treatment in which 43 mg N and 49.8 mg P per pot was applied a difference was found between the mycorrhizal and the nonmycorrhizal plants, while the phosphorus level was high and the percentage VAM infection was low. Therefore the results obtained by dry weight assessment should be interpreted with care.

				nitrogen level N-1		nitrogen level N-2	
				dry weight shoot (g)	% VAM	dry weight shoot (g)	% VAM
inoculation method I	phosphorus level P-2	+ VAM	mean	1.68	7	1.62	3
			s.d.	0.52	3	0.02	0
inoculation method II	phosphorus level P-1	- VAM	mean	0.97	0	0.88	0
			s.d.	0.06	0	0.05	0
		+ VAM	mean	1.18	69	0.99	57
			s.d.	0.06	9	0.08	7
	phosphorus level P-2	- VAM	mean	1.69	0	1.79	0
			s.d.	0.10	0	0.25	0
		+ VAM	mean	1.61	37	1.67	27
			s.d.	0.04	6	0.06	3

Table 6.3 Effect of inoculation method on VAM development in spring wheat, cv. Bastion, and the dry weight of shoots at two levels of nitrogen and phosphorus. Method I: inoculation with VAM-infected tomato roots banded in the pot; Method II: inoculation with inoculum-soil mixed through the pot. The figures are means of five replicates. The assessments were made on 30 days old plants.

6.3.2 *Experiment 2: The VAM development in relation to the inoculation method*

The results of this experiment are shown in table 6.3. When the mycorrhizal development at the highest phosphorus level (P-2) and at both nitrogen levels (N-1 and N-2) are compared with each other, it shows that inoculation with inoculum-soil (inoculation method II) gave a markedly better development of VAM in wheat than the inoculation with only infected roots (inoculation method I). The results obtained after inoculation with only infected roots were similar to those obtained in experiment 1; viz. a low percentage VAM at high phosphorus levels and only a small effect of various nitrogen levels. This indicates that in a soil that is rich in phosphorus the method of inoculation (equivalent to the distribution of inoculum in the soil) becomes a more important

factor in order to obtain a relatively high VAM infection level, compared to soil low in phosphorus. In this experiment the differences in VAM development and in dry weight of the shoots between the treatments with low and high nitrogen levels were rather small.

6.3.3 *Experiment 3: Effect of VAM on plant growth in relation to plant density and application of phosphorus*

The results obtained in this experiment are given in table 6.4. When phosphorus was deficient (P-1), inoculation of wheat with *G. mosseae* had a positive effect on plant growth at both plant densities. At the high phosphorus level (P-2), the mycorrhizas had a negative effect on plant growth. The plant density influenced the effect that plant density had on plant growth. The stimulatory effect at the low phosphorus level decreased with increasing plant density, while in the case of the high phosphorus level the negative effect was increased.

The nonmycorrhizal plants which had not been fertilized with phosphorus had a lower shoot-root ratio (S/R-ratio) than the plants in the other treatments. Application of phosphorus fertilizer enhanced the shoot growth more than the root growth, as VAM did also. The increase in S/R-ratio by additional P or by VAM tended to be larger at the low plant density. The dry matter content of the plants did not differ between the treatments.

As expected, the highest frequency of the mycorrhizas was found in the treatments without additional phosphorus. This corresponded also with the highest total length of the mycorrhizal roots per plant. The plants in the high density treatment were less mycorrhizal, both on basis of total length and percentage, than the plants in the low density treatment. That this would be the result of lower assimilation rates in the treatments with the high plant densities is not confirmed by the dry matter percentages of the plants.

	level of P fertilizer	number of plants per pot		dry weight shoots per plant (g)	dry weight roots per plant (g)	shoot/ root ratio	% dry matter	total root length (m per plant)	root density ($cm\ cm^{-3}$)	% VAM	infected root length (m per plant)	relative effect on growth
- VAM	P-1	2	mean	1.09	0.170	6.54	10.6	88.2	1.35	0	0	100
			s.d.	0.11	0.033	0.94		4.9				
+ VAM	P-1	2	mean	1.59	0.166	9.69	10.4	69.4	1.04	47	32.6	146
			s.d.	0.13	0.030	1.32		16.0		7		
- VAM	P-2	2	mean	2.35	0.278	8.49	10.2	122.3	1.84	0	0	215
			s.d.	0.08	0.114	0.60		24.7				
+ VAM	P-2	2	mean	2.19	0.226	10.04	9.3	55.7	0.84	18	10.0	201
			s.d.	0.19	0.060	2.01		11.7		4		
- VAM	P-1	12	mean	0.75	0.118	6.42	10.9	57.6	5.20	0	0	69
			s.d.	0.07	0.022	0.70		8.1				
+ VAM	P-1	12	mean	0.92	0.119	7.93	11.6	50.0	4.51	43	21.5	84
			s.d.	0.06	0.026	1.48		7.8		4		
- VAM	P-2	12	mean	1.54	0.193	8.15	11.8	79.7	7.19	0	0	141
			s.d.	0.13	0.043	1.32		16.6				
+ VAM	P-2	12	mean	1.31	0.175	7.60	11.1	92.9	8.38	10	9.3	120
			s.d.	0.04	0.028	1.21		30.1		2		

Table 6.4 Effect of two plant densities (2 and 12 plants per 13.3 kg soil) and use of phosphorus fertilizer equivalent to 0 kg (P-1) and 522 kg P_2O_5 ha^{-1} (P-2) on the growth response, shoot-root ratio, dry matter content, total root length, total length of VAM and the root density of mycorrhizal (Glomus mosseae) and nonmycorrhizal spring wheat plants cv. Bastion. Each treatment consisted of three replicates. The assessments were made on 46 days old plants.

6.4 DISCUSSION

6.4.1 *Relation between nutrient availability and VAM development*

Much evidence is available showing that plant nutrition affects the development of mycorrhizas. Most attention has been paid to the relation between phosphorus and VAM, but little attention has been paid to nitrogen in this respect (cf. Mosse, 1973; Smith, 1980). In this chapter, the results of experiment 1 show an interaction between nitrogen and phosphorus fertilizers. At the low phosphorus level, the VAM development first increased with increasing levels of nitrogen, but after an optimum was reached, higher nitrogen levels suppressed it. At the high phosphorus level the VAM development decreased over the whole range of nitrogen levels that were studied.

This kind of interaction had already been found by Björkman (1970) for ectomycorrhizas in *Pinus*. A number of VAM investigators mentioned in their discussions the role of a certain balance between nitrogen and phosphorus for optimal mycorrhizal development. The particular nature of this balance has not yet been defined. The observation of Hayman (1975) that under field conditions nitrogen had even more effect on the number of spores in the soil than phosphorus, may have been influenced by the nitrogen-phosphorus interaction. In experiment 1, in which the inoculum was banded in the soil, a larger VAM development in wheat was found in cases where only phosphorus was deficient as compared to situations in which only nitrogen was deficient. An explanation for this difference in spread of VAM in roots in cases of different types of deficiency might be found in an altered metabolism of the plant.

The VAM levels obtained in experiment 1 at the high phosphorus level and at various nitrogen levels were markedly lower than those found in the field at the same stage of plant development and at comparable levels of soil fertility (see chapter 5.3.1). The use of different inoculation methods

(experiment 2) indicated that the distribution of the inoculum through the soil is of importance. Especially in the short periods of plant growth, as do occur in our climatic zone, the amount and type of inoculum should also be taken into account (Powell, 1976; Hall, 1976; Carling et al., 1979 and Smith & Smith, 1981). In experiment 2 both aspects are involved and the results obtained in this experiment indicate that the amount and distribution of inoculum also account for the considerable difference in VAM development at the various nitrogen levels in the field.

The results obtained on the interaction between nitrogen and phosphorus has consequences for the interpretation of what generally is assumed as the relation between VAM and the nutrient availability. Björkman represented this relation graphically for the development of ectomycorrhizas in conifer plants and considered the development of mycorrhizas in relation to the growth of the trees (fig. 6.2). Mosse (1978) believed a similar pattern to be valid for endomycorrhizas as well (fig. 6.3).
Björkman's explanation of the observed relations was based on the carbohydrate levels in the roots, namely more carbohydrates give more mycorrhizas. Although his hypothesis received criticism (Lewis, 1975), it is in correspondence with the fact that the mycorrhizal fungi are ecologically dependent on the energy supplied by the host plant. Mosse attached much value to the dependence of the VAM development on internal nutrient (phosphorus) levels.

Our results show that the VAM development also depends on the interaction between nutrients (fig. 6.4). Based on this experience the hypothetical curve of Mosse is extended (fig. 6.5) by relating the VAM development with the weight of the host, as also Björkman (1970) did. Depending on the nutrient level of the substrate three different situations can be recognized. In fig. 6.5 the part A-B of the figure indicates nutrient levels that result in increasing VAM development at increasing nutrient levels together with higher yields. B-C shows a decrease of VAM development at increasing nutrient

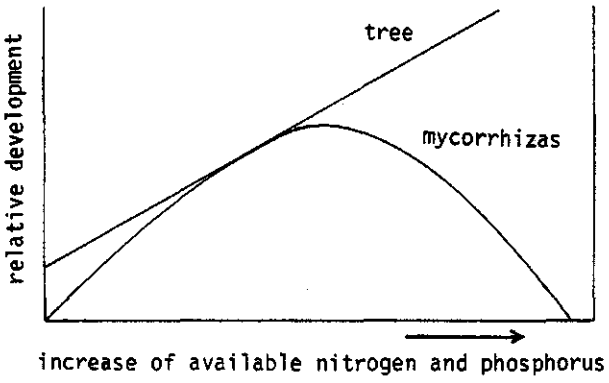

Figure 6.2 Relation between growth of conifer plants and the occurrence of ectotrophic mycorrhizae at increasing amounts of available nitrogen and phosphorus in the substrate (after Björkman, 1970).

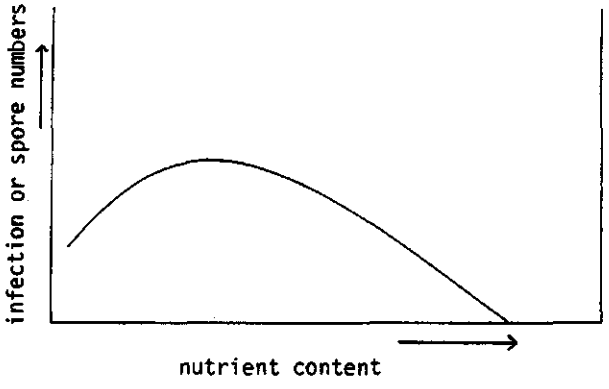

Figure 6.3 Effect of nutrient level on mycorrhizal infection (after Mosse, 1978).

levels also together with higher yields and C-D shows a decrease of VAM development at increasing nutrient levels together with lower yields. This picture is not a static one. It is thought to change with circumstances. The location of the points B and C (as projections of the top of the curves on the abcis), and their mutual distance is variable, indicating the interactions between the nutrients. The height of the top of the curves can change too, depending on the host plant, the temperature, soil and other factors influencing the plant growth. Although this way of representing the relation between VAM development, plant growth and nutrient availability contains speculations, it helps to give a better understanding of the apparently contradictory results obtained in work with mycorrhizal symbiosis.

6.4.2 *Relation between VAM development and the effect of VAM on the growth of (wheat) plants*

In order to obtain a growth stimulating effect of VAM it is necessary to have a sufficient amount of VAM infection. The occurrence of many mycorrhizal roots, however, does not always imply a stimulation of plant growth. This is shown in experiment 1 in which the presence of high VAM levels in wheat had hardly any positive effect on the growth. It is known that wheat, notwithstanding its finely branched root system, can respond markedly to VAM in the field (Khan, 1975). In experiment 1 four plants grew in 1.5 litre of soil, while Khan grew wheat plants at a density of about 30.000 ha^{-1} (= equivalent to 1 plant in 6.7 litre of soil). In relatively small amounts of substrate for rooting, the depletion zones of the individual roots can overlap each other. The role of the external hyphae of VAM as an auxiliary root system is therefore influenced by the root density. The results of experiment 3 illustrate this by showing a decrease of the growth promoting effect of VAM when plant density is increased.

In order to obtain, at different plant densities, comparable differences in root density, the plants in experiment 3 were given the same amount of nitrogen per plant. Phosphorus

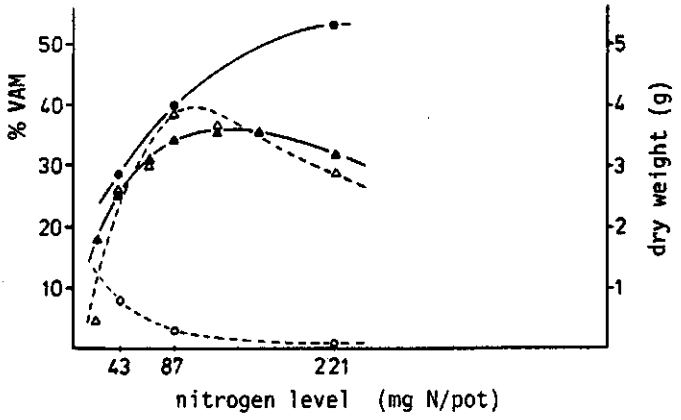

Figure 6.4 Effect of high (0,●) and low (Δ,▲) phosphorus levels on VAM development (----) and dry weight (———) of spring wheat plants at various levels of nitrogen. Data derived from table 6.2.

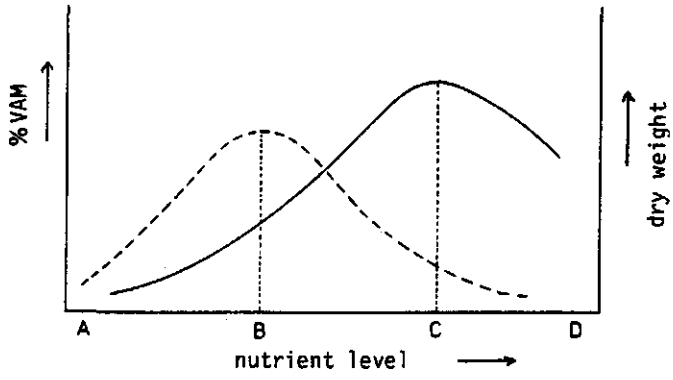

Figure 6.5 Effect of nutrient level on VAM development (----) in, and on the growth (———) of plants.

was applied, irrespective the plant density. It was assumed that because of the low mobility of phosphorus in the soil the root surface was the main limiting factor for P-uptake and not the total P-content per pot. The assumption, that all the nitrogen is available in the low density situation, may be an overestimation of the mobility of nitrogen in the soil, especially when N is available as NH_4^+-ions. The nitrogen fertilizer that was given per plant also led to a change of nitrogen-phosphorus ratio in the soil. As indicated earlier, this affects the VAM development in the roots and possibly affects also the microbial population in the soil and its activity. Nevertheless, the increase of plant density requires additional application of fertilizers. The results of experiment 3 can therefore not exclusively be the effect of plant density but approaches the effect how plant density, as an agricultural practice, can influence the effect of VAM on plant growth in the field.

The results of experiment 3 show also a growth increasing effect of VAM in the low phosphorus situation and a negative effect of VAM in the high phosphorus situation. Negative effects have been recorded several times (Crush, 1973, 1976; Cooper, 1975; Hall, 1977; Buwalda et al., 1982).
These negative effects are probably based on an excessive drain of carbon by the fungus (Buwalda et al., 1982). This drain of carbon leads presumably only to negative effects if the plant could still have used it for extra growth. The demand for energy of the fungus can be larger than what can be supplied by the plant and thus give rise to negative effects on plant growth. These situations can occur at low assimilation rates and at relatively large biomasses of mycorrhizal fungi. Irrespective of the causal mechanism, these can be assumed as cases in which the symbiosis is unbalanced.

7 *VAM AND PATHOGENIC FUNGI*

7.1 INTRODUCTION

Within the scope of this study it was of interest to investigate whether VAM interacted with diseases, and whether this interaction was influenced by disease management. As reviewed by Schenck & Kellam (1978) VAM can interact with plant pathogens. Schönbeck (1978) postulated that root pathogens and pathogens of aerial parts of the plants differ in their reaction upon the presence of mycorrhizas. He stated that, in general, the effect of root pathogens is decreased, but that of pathogens acting aboveground is often stimulated by VAM.

The experiments described in this chapter were of an exploratory nature and were mainly directed at three aspects: (1) The effect of the leaf pathogen *Puccinia recondita* on the mycorrhizal development in wheat, (2) the possible protection of wheat plants by VAM against attack by the root pathogen *Gaeumannomyces graminis* and (3) the effect of fungicides on the germination of the spores of *Glomus mosseae*.

7.2 MATERIALS AND METHODS

7.2.1 *Soil*

The light calcareous clay loam, described as soil B in 6.2 and characterized in table 6.1, was used in all experiments. Each pot contained 700 g of dry soil, which was covered with fine gravel after transplanting the seedlings. The moisture content was kept at about 60 percent of the upper plasticity limit. The pots in the germination test contained 500 g of dry soil. The soil in which plants were grown received 10 mg N per plant given in the form of ammonium nitrate.

7.2.2 *Host plant*

Seedlings of spring wheat, cv. Bastion were used. Seeds, disinfested with 2% NaClO, were allowed to germinate in autoclaved sand. After two to three days the seedlings were transplanted, 10 per pot in the experiments with *P. recondita* and 3 per pot in the experiment with *G. graminis*.

7.2.3 *Inoculation with Glomus mosseae*

The inoculum used consisted of finely cut potballs of mycorrhizal tomato plants. In the *P. recondita* experiment it was mixed with the soil in ratios of 1:20; 1:10; 1:5. The number of diaspores of the endophyte in the 1:10 dilution was estimated at about 12 diaspores g^{-1} soil. In the other experiments a 1:12 dilution was used. Autoclaved inoculum-soil was used as control. The inoculation was done simultaneously with the filling of the pots.

7.2.4 *Inoculation with Puccinia recondita*

The wheat seedlings were inoculated with the brown rust fungus at six and fourteen days after transplanting or only once at 10 days after transplanting. After inoculation with the *P. recondita* spores, the plants were incubated for 24 hours in the dark at about 100% relative humidity.

7.2.5 *Defoliation of plants*

The reduction of the green leaf area by *P. recondita* was simulated by defoliation of wheat plants. This was done ten days after transplanting the seedlings. The first two leaves (the expanded leaves) were cut off.

7.2.6 *Inoculation with Gaeumannomyces graminis*

Two different isolates (isolate L and F) were used. They were derived from infected plants in the field by means of single ascospore cultures. The inoculum was obtained by growing the fungi in a mixture of wheat grains and finely cut

straw as described by Gerlagh (1968). The amount of inoculum used, was in the order of one and three gram of inoculum per pot. Inoculation with the pathogen and with *G. mosseae* were carried out simultaneously.

7.2.7 *Assessment of disease*

For disease assessment of *G. graminis* an index (0-3) was used of which the value depended on the distance of the lesions from the attachment of the root. In case the latter distance was 0-4, 4-8 or more than 8 cm, an index of 3, 2 or 1 was given respectively, and a zero in case there was no lesion at all.

7.2.8 *Spore germination test*

The spores used, were obtained from sporocarps of *Glomus mosseae* which had been stored three weeks at 5 °C. Each replicate consisted of 25 spores, selected at random, which were placed between two membrane filters (Selectron 0.2 µm, 47 mm in diameter) and kept together between two perspex rings. The filters were buried in soil B (table 6.1) and incubated for two weeks at room temperature. The germination of the spores was scored using a dissecting microscope after staining each spore with a little drop of 0.05% trypan blue in lactophenol.

7.2.9 *Fungicides*

Six fungicides from different chemical groups were used. The fungicides were: (1) benomyl (methyl 1-(butylcarbamoyl) benzimidazol-2-ylcarbamate) as Benlate 50%; (2) zineb (zinc ethylenebisdithiocarbamate) as Shell Zineb 70%; (3) metalaxyl (D,L-N-(2,6-dimethylphenyl)-N-(2'-methoxyacetyl)-alanine-methyl-ester as Ridomil 25%; (4) triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)-2-butan-2-one) as Bayleton 25%; (5) quintozene (pentachloronitrobenzene) as Liro-PCNB-50% (6) carbendazim/maneb (methyl benzimidazol-2-ylcarbamate/manganese ethylenebisdithiocarbamate) as Bavistin M (6/50%). They were applied in three concentrations viz.

10, 1 and 0.1 times the concentration advised for practical application.

7.3 RESULTS

7.3.1 *Interaction between Puccinia recondita and VAM*

In a preliminary experiment in which the leaves of spring wheat were inoculated twice with the brown rust fungus, about 38 percent of the total leaf area became covered with rust lesions. This resulted in a decrease of the fresh weight of the roots of the rust inoculated plants (table 7.1). Rust infected plants that were inoculated with *Glomus mosseae* had a higher percentage of VAM than mycorrhizal plants that were not inoculated with the rust fungus. Thus inoculation of wheat plants with *Puccinia recondita* stimulated the relative development of the mycorrhizas. On the other hand the presence of VAM did not influence the rust development in the leaves.

In order to investigate the nature of the VAM stimulation by *P. recondita* two additional experiments were carried out viz. an experiment in which the plants in half of the treatments were defoliated and a similar experiment in which the plants in half of the treatments were inoculated with *P. recondita*.

	- VAM				+ VAM					
	- P. recondita		+ P. recondita		- P. recondita			+ P. recondita		
	fresh weight roots (g)	% rust	fresh weight roots (g)	% rust	fresh weight roots (g)	% rust	% VAM	fresh weight roots (g)	% rust	% VAM
mean	6.2	0	5,4	37.7	6.2	0	23.8	4.9	38.1	35.7
s.d.	0.6	0	0.7	5.3	0.6	0	4.3	0.6	6.3	5.6

Table 7.1 Effect of Puccinia recondita on VAM development and fresh weight of the roots of spring wheat plants, cv. Bastion. The figures are a mean of six replicates.

In the first of these two experiments (table 7.2), the reduction of the assimilation area of wheat by a leaf pathogen was similated by defoliation. Two weeks after defoliation a marked inhibition of root development could be found. At five weeks after defoliation this effect was still noticeable. The corresponding percentage of VAM was significantly higher in the roots of defoliated plants (analysis of variance, $p<0.05$). The marked influence of the amount of inoculum on the development of VAM was best observed two weeks after defoliation.

In the second of the two experiments (table 7.3), which was started one week later, the wheat plants were inoculated with *P. recondita*. This was done only once, in order to obtain a moderate infection. This made it possible to observe whether the rust fungus had a direct effect on the VAM development or not. After two weeks of incubation the rust fungus had exerted a slight detrimental effect on shoot and root weight. The percentage of VAM was not affected by the rust fungus, but only by the different amounts of VAM inoculum. After five weeks the inoculation of *P. recondita* had still a slight negative effect on shoot and root weights. At this latter observation time the VAM development was better correlated with the reduced root growth than at two weeks after rust inoculation.

Again, the presence of VAM had no effect on the rust development in the wheat leaves, when + and - VAM plants are compared at the same level of VAM inoculum. The amount of VAM inoculum, alive as well as dead, did have an effect on the rust.

From these results it became clear that the stimulating effect of the brown rust on the percentage VAM in wheat plants was probable primarily based on reduced root growth.

7.3.2 *Interaction between Gaeumannomyces graminis and VAM*

In preliminary experiments that were carried out, some evidence was found that the application of phosphorus in P-deficient soil reduced the pathogenic effect of *G. graminis* in

			+ VAM						- VAM					
inoculum density[1]			1:20		1:10		1:5		1:20[3]		1:10[3]		1:5[3]	
treatment[2]			D	ND	D	ND	D	ND	D	ND	D	ND	D	ND
2 weeks	root dry	mean	0.10	0.27	0.09	0.24	0.07	0.21	0.11	0.31	0.14	0.33	0.13	0.28
after	weight (g)	s.d.	0.03	0.04	0.01	0.02	0.01	0.03	0.01	0.04	0.01	0.04	0.03	0.01
defolia-	shoot dry	mean	0.36	0.86	0.28	0.75	0.29	0.78	0.31	0.88	0.31	0.86	0.34	0.82
tion	weight (g)	s.d.	0.04	0.06	0.03	0.03	0.03	0.04	0.03	0.06	0.04	0.04	0.05	0.04
	% VAM	mean	23.7	15.5	31.8	21.9	38.7	27.4	0	0	0	0	0	0
		s.d.	3.3	1.7	3.4	3.7	3.8	2.0						
5 weeks	root dry	mean	0.27	0.37	0.27	0.42	0.25	0.42			0.14	0.34		
after	weight (g)	s.d.	0.01	0.03	0.06	0.05	0.06	0.05			0.05	0.04		
defolia-	shoot dry	mean	1.62	2.79	1.52	2.47	1.33	2.38			0.98	2.09		
tion	weight (g)	s.d.	0.09	0.14	0.04	0.14	0.09	0.14			0.23	0.08		
	% VAM	mean	52.9	43.9	54.3	42.8	61.0	46.8			0	0		
		s.d.	2.9	4.9	7.0	2.4	4.1	2.4						
% VAM at defoliation			5.2		8.8		18.6							

Table 7.2 Effect of defoliation on VAM development and on dry weights of spring wheat plants (cv. Bastion).

[1] Glomus mosseae was inoculated as inoculum-soil in the given ratios.

[2] D = defoliated plants; ND = non defoliated plants.

[3] Autoclaved inoculum soil was used.

			+ VAM						- VAM					
inoculum density[1]			1:20		1:10		1:5		1:20[3]		1:10[3]		1:5[3]	
treatment[2]			R	NR	R	NR	R	NR	R	NR	R	NR	R	NR
2 weeks after rust inoculation	% leaf area covered with rust lesions	1st leaf	8.8		11.8		17.8		13.4		12.2		18.6	
		2nd leaf	2.6		4.6		5.6		5.8		3.0		7.0	
	root dry weight (g)	mean	0.15	0.16	0.12	0.15	0.11	0.17	0.12	0.18	0.15	0.21	0.16	0.23
		s.d.	0.02	0.03	0.02	0.02	0.02	0.04	0.02	0.02	0.02	0.02	0.05	0.04
	shoot dry weight (g)	mean	0.63	0.74	0.58	0.71	0.63	0.74	0.65	0.78	0.56	0.79	0.60	0.79
		s.d.	0.03	0.06	0.08	0.04	0.05	0.05	0.02	0.06	0.08	0.06	0.10	0.03
	% VAM	mean	17.4	17.8	23.5	22.4	30.0	32.5	0	0	0	0	0	0
		s.d.	2.2	3.0	3.3	2.7	2.5	3.7						
5 weeks after rust inoculation	root dry weight (g)	mean	0.39	0.42	0.35	0.49	0.45	0.47			0.40	0.58		
		s.d.	0.01	0.03	0.04	0.04	0.08	0.05			0.05	0.05		
	shoot dry weight (g)	mean	1.93	2.33	1.89	2.35	2.04	2.19			2.15	2.58		
		s.d.	0.06	0.05	0.12	0.03	0.25	0.09			0.19	0.19		
	% VAM	mean	50.7	40.1	51.1	43.2	46.3	40.2			0	0		
		s.d.	2.4	3.1	2.3	2.6	5.2	5.0						
% VAM at rust inoculation			11.9		15.1		26.1							

Table 7.3 Effect of inoculation with Puccinia recondita (brown rust) on VAM development and on dry weights of spring wheat plants (cv. Bastion).

[1] Glomus mosseae was inoculated as inoculum-soil in the given ratios.

[2] R = rust inoculated plants; NR = plants not inoculated with rust

[3] Autoclaved inoculum soil was used.

A			% VAM	disease index	shoot length (cm)	shoot dry weight (g)
16 mg P	isolate L	+ VAM	5.1	2.22	29.8	0.237
		- VAM	0	2.48	26.2	0.193
	isolate F	+ VAM	7.0	1.83	29.3	0.230
		- VAM	0	1.87	28.8	0.227
	control	+ VAM	18.7	0.88	36.2	0.370
		- VAM	0	0.77	34.1	0.307
8 mg P	isolate L	+ VAM	9.7	2.14	26.1	0.207
		- VAM	0	2.43	26.6	0.140
	isolate F	+ VAM	6.0	1.60	27.3	0.190
		- VAM	0	2.41	27.0	0.190
	control	+ VAM	24.5	0.90	36.0	0.340
		- VAM	0	1.18	35.0	0.293
0 mg P	isolate L	+ VAM	7.6	2.22	27.4	0.187
		- VAM	0	2.40	25.3	0.150
	isolate F	+ VAM	6.3	2.30	24.1	0.123
		- VAM	0	2.14	26.2	0.170
	control	+ VAM	18.0	0.70	35.9	0.343
		- VAM	0	1.27	30.4	0.227

B			% VAM	disease index	shoot length (cm)	shoot dry weight (g)
16 mg P	isolate L	+ VAM	3.7	2.34	25.3	0.167
		- VAM	0	2.67	24.3	0.120
	isolate F	+ VAM	4.8	2.06	24.6	0.183
		- VAM	0	2.47	24.4	0.147
	control	+ VAM	15.7	0.93	34.4	0.360
		- VAM	0	0.95	33.0	0.300
8 mg P	isolate L	+ VAM	4.6	2.58	23.8	0.153
		- VAM	0	2.33	21.6	0.117
	isolate F	+ VAM	6.5	1.95	27.0	0.200
		- VAM	0	2.64	23.7	0.133
	control	+ VAM	19.2	0.80	36.3	0.353
		- VAM	0	1.66	36.7	0.313
0 mg P	isolate L	+ VAM	5.4	2.16	20.4	0.110
		- VAM	0	2.51	22.4	0.107
	isolate F	+ VAM	8.6	2.28	24.6	0.160
		- VAM	0	2.28	23.6	0.130
	control	+ VAM	25.4	0.65	33.9	0.263
		- VAM	0	0.96	35.0	0.270

Table 7.4 Interaction between Glomus mosseae, two isolates of Gaeumannomyces graminis and P-fertilizer measured as effects on disease index, shoot length and shoot dry weight of 24 days old spring wheat plants cv. Bastion.
Two inoculum potentials of G. graminis were used, namely 1 g (A) or 3 g (B) of inoculum per pot.

wheat. This agreed with observations done at the Rothamsted Experimental Station (Mattingly et al., 1980). In the same preliminary experiments it was also observed that VAM had a negative effect on the growth of wheat plants in the presence of a relatively high concentration of inoculum of *G. graminis*. This effect was not found at lower inoculum potentials of the pathogen. Nitrogen fertilizers increased plant weight but did not modify the interaction between the pathogen and VAM in relation to the host plant.

Based on the above described observations, another experiment was carried out of which the results are given in the tables 7.4 and 7.5. In this experiment the interaction between VAM and two isolates of *G. graminis* was studied at three levels of application of phosphorus (0, 8 and 16 mg P per pot) and at two levels of inoculum of the pathogen.

In most treatments the VAM plants had a lower disease index. These differences were shown to be highly significant (table 7.5). Application of phosphorus had no significant effect on the disease index. In accordance with its larger pathogenicity isolate L gave a higher disease index than isolate F.

In all treatments with *G. graminis* the VAM development was far less than in those of the control. No significant difference was found between the two isolates of the pathogen in this respect, except for the fact that the high inoculum level isolate L tended to cause a lower percentage of VAM

Source	disease index	shoot length	shoot weight
P effect	1.18	4.12*	14.42**
VAM effect	18.70**	2.17	23.64**
isolate effect	7.57**	0.87	2.76
effect of inoculum level	4.88*	12.53**	23.10**
interaction P x VAM	2.72	0.12	0.44

Table 7.5 Analysis of variance of the data given in table 7.4.
The figures are F-values.

* = significant at $p<0.05$

** = significant at $p<0.01$

than isolate F. There was a reduction of the VAM development when more phosphorus was applied although this was not always prominent in all the treatments at the low inoculum level of *G. graminis*.

The inoculation of wheat plants with *G. graminis* had a large negative effect on the dry weight of the shoots. The isolates differed little in their effect on plant growth. The inoculum potential was more important in this respect. The detrimental effect of the pathogen was diminished by the application of phosphorus as well as by inoculation of the plants with *Glomus mosseae*. In this experiment the stimulating effect of phosphorus on the plant growth was similar to the effect of VAM. Both effects were independent and therefore additive.

The measurements of shoot length gave results very similar to those of the shoot weights.

7.3.3 *The germination of spores as affected by fungicides*

As shown in table 7.6 the germination of the spores of *G. mosseae* was influenced by fungicides. The type of fungicide and the applied concentration were of importance. The most prominent effects were obtained with zineb and quintozene at the concentration of ten times the advised dose. In the case of zineb, at all the used concentrations the spore

		spore germination as percentage of the control			
fungicide	concentration	10x	1x	0.1x	concentration in soil at 1x (ppm a.i.)
zineb		5.5*	91.4	73.0	3.5
benomyl		69.9	43.8*	80.4	0.25
metalaxyl		76.7	72.9	87.7	0.25
quintozene		31.1*	77.8	78.5	15
triadimefon		65.8	85.9	100.3	0.125
carbendazim/maneb		52.7	142.0	94.7	2.24

Table 7.6 Germination of Glomus mosseae spores (as percentage of the control) as affected by various fungicides applied to the soil at concentrations 10, 1 and 0.1 times the dose used in practice as foliar applications.
The amount of usually foliar applied fungicide per area was mixed with a 10 cm soil layer.
* = significant different from control ($p<0.05$; Wilcoxon-test).

germination was of a different type than it was the case in the control treatment. Metalaxyl and triadimefon had no significant effect at the concentrations used. Benomyl gave only a reduction of the spore germination at the advised dose and not at the higher and lower concentrations applied. Carbendazim/maneb stimulated the spore germination at 2.24 ppm but this was not significant.
In general, the effect of the tested fungicides on the germination of the spores of *Glomus mosseae* appeared to be rather small at concentrations used in common practice.

7.4 DISCUSSION

7.4.1 *Interaction between VAM and plant pathogens*

The compilation of interactions between VAM and plant diseases as given by Schenck & Kellam (1978) indicated that variable results have been obtained. The statement of Schönbeck (1978), viz. that VAM induce resistance against plant pathogens in the below ground parts of the plant and give enhanced susceptibility in the aerial part, does not seem to hold in all cases. A number of reports even gave an increasing effect of VAM on the disease severity of root pathogens (Baltruschat, 1975; Ross, 1972; Davis et al., 1979).

Our results showed no effect of VAM on the development of *Puccinia recondita* in wheat leaves. The effect of VAM on wheat inoculated with *Gaeumannomyces graminis* depended on the experimental conditions.

The mechanisms that are involved in these interactions are not yet fully understood. Nevertheless, three possible modes of action can be distinguished:
(1) Improved nutrition of the plant.

It is often difficult to separate the effect that increased nutrient uptake by VAM has, from the effect caused only by its presence only. As shown by Davis & Menge (1980), phosphorus fertilizer decreased the effect of *Phytophthora parasitica* in citrus as did inoculation with the VAM fungus *Glomus fasciculatus*. In cotton, phosphorus fertilizer increased the injury due to *Verticillium dahliae*, which also occurred after

VAM inoculation (Davis et al., 1979). These examples indicate that the use of phosphorus fertilizers may have a similar effect on the disease incidence as inoculation with VAM.

The decreased effect of the pathogen might be based on compensation for the decreased nutrient uptake by the diseased root system. This can happen by giving extra phosphorus or by the enlargement of the root system through the extramatrical VAM mycelium. Evidence for this can be found in the results of Chou & Schmitthenner (1974), Schönbeck & Dehne (1977) and Zambolin & Schenck (1980a, b) in which VAM did not affect the disease level but did have a positive effect on the growth of the plants. Similar results were obtained with the effect of phosphorus on wheat attacked by *G. graminis* (table 7.5).

Some evidence is available that increased phosphorus uptake decreases the permeability of the root cell membranes of the host plant (Ratnayake et al., 1978). This phenomenon may be correlated with reduced disease incidence caused by *G. graminis* in mycorrhizal wheat (Graham & Menge, 1982). The results, described in 7.3.2, showed no effect of phosphorus on the disease index as VAM did. This is not in agreement with their explanation that in the case of phosphorus application reduced leakage of root exudates decreases the activity of the pathogen.

(2) Effect on disease resistance c.q. susceptibility.

This mechanism is thought to act mainly by changes in the physiology of the host that results in, for example, the lignification of cell walls (Joseph, 1977), the larger vascular volumina (Daft & Okusanya, 1973; Joseph, 1977), and changes in the composition of amino acids in the plant (Schönbeck & Dehne, 1977). However, similar effects can also be obtained by improved nutrition or by other aspecific avirulent invaders (Matta, 1981; Kuć, 1981). The observed effects are only correlations with disease development and cannot be considered as direct evidence for the mechanism involved. This is because of the fact that intertwining with the effect of VAM on the nutrition often occurs.

(3) Competition for the same ecological site.

The protective effect of VAM occurs mainly at the location of the mycosymbiont (Schönbeck & Dehne, 1979), or at least when a good mycorrhizal establishment is present (Bärtschi et al., 1981). This suggests that the protective mechanism can be based on direct competition for the site. However, this will probably never happen to the same degree as with ectomycorrhizas (Marx, 1972). The physical barrier, formed by the fungal sheath present in these mycorrhizas is lacking in the case of VAM. The site occupied by the pathogen needs to be very similar to that of the VAM fungus before competition for the same niche can be expected. The specialized parasite *G. graminis* which has a limited competitive saprophitic ability and forms hyphae along the roots may be such a fungus. The results in the experiment in which VAM reduced the disease development, independent of the phosphorus nutrition, may point in this direction.

7.4.2 *Effect of biocides on VAM*

Within the scope of this study the use of biocides as crop protection chemicals was of importance. Results of studies on the effect of these on VAM appeared not easily to be translated to field situations. As indicated by the results of Nesheim & Linn (1969) phytotoxic effects of the chemicals used, have to be taken into account. In many papers, however, proper controls are missing. In most cases, comparison with a nonmycorrhizal control cannot be made. This renders it impossible to recognize possible phytotoxic effects. Even when the recommended dose is used, these controls are still obligatory.

Beside the effect on the symbiosis, an effect of the biocides on the pre-infection phase of the mycorrhizal fungi can be expected. The results of the test of the germination of *G. mosseae* spores (table 7.6) gave only slight effects of these fungicides at the recommended dose (note the assumption made as given in the text of table 7.6). With other biocides, Tommerup & Briggs (1981) obtained similar effects with three

other fungi. Jalali & Domsch (1975) showed that benomyl and triadimefon negatively influenced the VAM development in wheat.

Nevertheless it is necessary to realize that under conditions in which VAM fungi are involved in nutrient uptake (generally in low input agriculture) the use of biocides for crop protection will be limited. In high input agriculture the effect of VAM on plant growth is limited. There may be some exceptions to this like the citrus nurseries in California.

8 *CONCLUSIONS AND GENERAL DISCUSSION*

The occurrence of VAM fungal spores on the DOB-farms was investigated in the last year of the DOB-farm system in 1978. Soil samples collected over the previous 26 years of these farms were also examined for the presence of VAM fungal spores. The various farming systems used on the DOB-farms which differed in the application of organic matter at comparable and relatively high fertility levels, appeared to have no influence on the occurrence of VAM fungal spores.

In the new OBS-project, the development of VAM in winter wheat and potatoes was assessed. Already in the first year of this project, a remarkable development of VAM in winter wheat on the BD-farm was observed in comparison with its development on the GA- and GI-farm. In the subsequent two years this difference, which almost certainly resulted from nitrogen deficiency on the BD-farm, increased. The differences in VAM development between the farms were smaller in the potato crops. Nitrogen deficiency did not affect the development of VAM in this crop.

From the results obtained on the Lovink-farm, it can be inferred that the effect of various agricultural practices on the VAM development in winter wheat and potatoes can be attributed primarily to differences in the availability of nutrients and the VAM inoculum potential. The VAM development in wheat was negatively correlated with the yield of the crop.

Greenhouse experiments showed that the amount of available phosphorus interacted with the effect that nitrogen fertilizers had on VAM in wheat.

Although the VAM fungi isolated from soil of the DOB/OBS-farms stimulated growth of red clover plants in nutrient-deficient soil, there was no evidence that VAM stimulated growth of plants grown in the relatively rich soil on the farms. In estimating the significance of VAM on the growth of plants in various situations, it should be remembered that

mycorrhizal symbiosis has both a positive and negative effect on plant growth. This view on the mycorrhizal symbiosis contributes to a better understanding of the opposite effects which can be obtained with VAM, as for example in the plant density experiment (chapter 6). Whether the observed effect on plant growth is ultimately positive (mutualism) or negative (parasitism) depends on the importance of each of the two opposing effects.

In natural ecosystems factors which affect the symbiosis, such as host plant species, availability of nutrients and plant density, are generally more or less constant and the positive effect of the symbiosis often prevails. In agriculture, the factors which affect symbiosis frequently change and therefore the balance between the positive and the negative effect is disturbed. It will take time to reach a new equilibrium, but before this is reached, transitory depressions in the growth of mycorrhizal plants by parasitism may occur (cf. S.E. Smith, 1980). Nevertheless, the dependence of VAM fungi on the energy supplied by their host plant together with a limited virulence implies that only small growth depressions will occur. There is no evidence available to suggest that VAM fungi can act as real pathogens as has been found for other mycorrhizal fungi, such as in vanilla (Alconero, 1969) and in marsh orchid (Downie, 1957).

Furthermore, the external mycelium of VAM acts predominantly as an auxiliary absorbing system for the plant. Thus VAM will improve plant growth under conditions in which the extent of the root system is the limiting factor for growth, and in which the soil has not been fully depleted of nutrients, especially those nutrients with limited mobility in the soil. This implies that high levels of VAM development can be present without having a substantial effect on plant growth (see chapter 6.3, experiment 1). Development of VAM in the roots of plants occurs when the plant is a suitable host for the fungus and the invading capacity of the fungus is sufficient. Whether VAM promotes plant growth depends on additional environmental factors such as the presence of soil which has not been fully depleted of nutrients by the roots them-

selves. Thus an assessment of the percentage of VAM will only provide limited information on the absorption of nutrients by the fungus. Moreover, reduced root growth appeared to result in an increased percentage of VAM (see chapter 7.3.1) and this can obscure the real importance of VAM in the uptake of nutrients. Therefore measurements are required of the extent of VAM fungal mycelium outside the roots, and if possible, outside the depletion zone of the root.

The capability of VAM to protect the roots against pathogens partly results from improved nutrition of the plants. If, however, other mechanisms are involved, as for example an increased lignification of the cell walls, the level of VAM infection appears to be positively correlated with the degree of protection (Schönbeck & Dehne, 1979). The protection provided by VAM against root pathogens will therefore be most prevalent in nutrient-deficient soils which provide the best conditions for the development of VAM.

Advocating agricultural measures to improve the development of VAM or the promoting effect of VAM on plant growth in current Dutch agriculture does not seem to be realistic at present. This would imply an advocating of farming conditions in which the availability of plant nutrients is the limiting factor on plant growth. Where such conditions occur, however, it is desirable to consider how agricultural practices could affect the occurrence and significance of mycorrhizal fungi.

SUMMARY

The development and significance of vesicular-arbuscular mycorrhizas (VAM) in wheat and potatoes have been studied in relation to various farming systems and agricultural practices. The effects of farming systems on VAM have been observed on three neighbouring experimental farms in the vicinity of Nagele, in the North East Polder, The Netherlands. It appeared that varying amounts of organic matter used during a period of 26 years had little effect on the presence of VAM fungal spores. The results of a new project started on these farms in 1979 showed that nitrogen deficiency greatly stimulated the development of VAM in wheat. In potatoes, however, no such results were found. After winter wheat the number of spores of *Glomus mosseae* dominated the number of spores of the other species in the soil. After potatoes spores of *Glomus macrocarpus* were found more frequently.

The influence of various agricultural practices on the development of VAM has been studied in long-term field experiments on the Lovink-farm, another experimental farm in the North East Polder. The relation between VAM and the amounts of nitrogen fertilizers applied has also been assessed in these experiments. From the results it can be concluded that the effect of various agricultural practices on VAM is based primarily on the effect of these practices on the growth of the plants and the inoculum potential of VAM in the soil. In addition, it appears that the effect of nitrogen on the development of VAM depends on the host plant species involved. This is in accordance with the results obtained on the farms at Nagele.

Greenhouse experiments with wheat showed a strong interaction between the application of nitrogen and phosphorus fertilizers in relation to the development of VAM. When sufficient phosphorus was applied, the effect of nitrogen on VAM development depended on the way in which the VAM inoculum was applied.

The significance of VAM fungi for the growth of plants depends largely on the environmental conditions in which the plants grow, particularly the nutrition of the plants, plant density and inoculum potential of VAM. These factors largely determine whether the symbiosis results in a positive or even a negative effect on the growth of plants.

In spite of the fact that VAM fungi occur in the relatively rich arable soils in these polders, and that VAM fungi isolated from these soils greatly stimulate the growth of red clover in a poor soil, VAM does not appear as yet to be important for Dutch agriculture. Favourable conditions for the development of these mycorrhizas and conditions under which they have the greatest effect on the growth of plants are, in general, not present. Nevertheless, in circumstances in which little or no additional plant nutrients are used, or nutrients are not readily available for the plants, the importance of these fungi for the growth of plants increases.

Protection given by VAM to plants against root pathogens seems to be limited. It is predominantly based on improved nutrition of poorly-fed plants and in a few special cases on competition for a site inside or outside the mycorrhizal root.

SAMENVATTING

De ontwikkeling en de betekenis van vesiculair-arbusculaire mycorrhiza's (VAM) in tarwe en aardappel werd bestudeerd met betrekking tot verschillende landbouwsystemen en de toepassing van diverse cultuurmaatregelen.

De waarnemingen betreffende het effect van landbouwsystemen op VAM werden verricht op drie naast elkaar gelegen proefbedrijven te Nagele (Noordoostpolder). Hierbij bleek dat een 26 jaar lange toepassing van verschillende hoeveelheden organisch materiaal in de drie bedrijfssystemen van weinig invloed is geweest op het voorkomen van VAM. In het inmiddels op deze bedrijven nieuw gestarte project, waarin de ontwikkeling van bedrijfssystemen centraal staat, bleek dat stikstofdeficientie de ontwikkeling van VAM in tarwe sterk stimuleerde. Een dergelijk beeld werd niet in het gewas aardappel aangetroffen. Na wintertarwe domineerden de sporen van *Glomus mosseae* in de grond, na aardappel die van *Glomus macrocarpus*.

De invloed van cultuurmaatregelen op de ontwikkeling van VAM werd bestudeerd op de Lovink-hoeve, een proefboerderij te Marknesse (Noordoostpolder). Hierbij werd tevens de relatie tussen de ontwikkeling van VAM en de gebruikte hoeveelheid stikstof meststof onderzocht. Op grond van de verkregen resultaten werd geconcludeerd dat de invloed van de diverse cultuurmaatregelen in eerste instantie gebaseerd is op de invloed van deze maatregelen op de groei van het gewas en het inoculumpotentiaal van VAM in de grond. Tevens bleek dat het effect van stikstof op de ontwikkeling van VAM afhankelijk is van de waardplantsoort. Dit is in overeenstemming met de resultaten die verkregen werden op de bedrijven in Nagele.

In kasexperimenten met tarwe werd er met betrekking tot VAM gevonden dat er een sterke interactie bestaat tussen de stikstof- en fosfaatbemesting. Bij een voldoend hoog fosfaatniveau was het effect van stikstof op de ontwikkeling van VAM afhankelijk van de wijze van toedienen van het VAM inoculum.

De betekenis van de VAM schimmels voor de groei van planten is sterk afhankelijk van de omstandigheden waaronder de plant groeit, waarbij vooral de voeding, de plantdichtheid en het inoculumpotentiaal van VAM van belang zijn. Er werd aangetoond dat het mede van deze factoren afhankelijk is of de symbiose met de schimmel tot een positief of zelfs tot een negatief effect op de groei van de plant leidt.

Ondanks het algemeen voorkomen van de VAM schimmels in de rijke landbouwgronden van de polders en het feit dat deze schimmels in een arme grond de groei van rode klaver sterk stimuleerden, lijkt de betekenis van VAM voor de Nederlandse landbouw vooralsnog gering te zijn. De omstandigheden, waaronder deze mycorrhiza's zich sterk ontwikkelen en een bijdrage leveren tot een betere groei van het gewas is in de regel niet aanwezig. Naarmate er echter minder gebruik gemaakt wordt van meststoffen of wanneer de beschikbaarheid ervan geringer is, zal de betekenis van deze schimmels voor de groei van planten toenemen.

De beschermende werking van VAM tegen wortelpathogenen lijkt beperkt te zijn. Wanneer er bescherming optreedt, berust dit voornamelijk op een grotere weerstand van de plant door een verbetering van de voeding of op competitie tussen VAM schimmels en het pathogeen bij het innemen van een plaats in en om de plantewortel.

REFERENCES

Abbott, L.K. and A.D. Robson, 1977. The distribution and abundance of vesicular-arbuscular endophytes in some Western Australian soils. Australian Journal of Botany 25: 515-522.

Abbott, L.K. and A.D. Robson, 1979. A quantitative study of the spores and anatomy of mycorrhizas formed by a species of Glomus, with reference to its taxonomy. Australian Journal of Botany 27: 363-375.

Alconero, R., 1969. Mycorrhizal synthesis and pathology of Rhizoctonia solani in vanilla orchid roots. Phytopathology 59: 426-430.

Ambler, J.R. and J.L. Young, 1977. Techniques for determining root length infected by vesicular-arbuscular mycorrhizae. Soil Science Society of America Journal 41: 511-555.

Azcon, R. and J.A. Ocampo, 1981. Factors affecting the vesicular-arbuscular infection and mycorrhizal dependency of thirteen wheat cultivars. New Phytologist 87: 677-685.

Bakermans, W.A.P. and C.T. de Wit, 1970. Crop husbandry on naturally compacted soils. Netherlands Journal of Agricultural Science 18: 225-246.

Baltruschat, H., 1975. Untersuchungen über den Einfluss der endotrophen Mycorrhiza auf den Befall von Pflanzen durch pathogene Pilze, insbesondere von Nicotiana tabacum durch Thielaviopsis basicola. Dissertation Rheinischen Friedrich-Wilhelms-Universität, Bonn: 137 pp.

Bärtschi, H., V. Gianinazzi-Pearson and I. Vegh, 1981. Vesicular-arbuscular mycorrhiza formation and root rot disease (Phytophthora cinnamomi) development in Chamaecyparis lawsoniana. Phytopathologische Zeitschrift 102: 213-218.

Bary, A.M. de, 1879. Die Erscheinung der Symbiose, Strassburg.

Baylis, G.T.S., 1970. Root hairs and phycomycetous mycorrhizas in phosphorus-deficient soil. Plant and Soil 33: 713-716.

Baylis, G.T.S., 1972. Minimal levels of phosphorus for nonmycorrhizal plants. Plant and Soil 35: 233-234.

Baylis, G.T.S., 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. In: Endomycorrhizas. Eds. F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press, London: 391-407.

Bertheau, Y., V. Gianinazzi-Pearson et S. Gianinazzi, 1980. Développement et expression de l'association endomycorrhizienne chez le blé. I. mise en évidence d'un effet variétal. Annales de l'Amélioration des Plantes 30 (1): 67-78.

Bevege, D.I., 1968. A rapid technique for clearing tannins and staining intact roots for detection of mycorrhizas caused by Endogone spp., and some records of infection in Australasian plants. Transactions of the British Mycological Society 51: 808-810.

Björkman, E., 1970. Forest tree mycorrhiza - The conditions for its formation and the significance for tree growth and afforestation. Plant and Soil 32: 588-610.

Black, R.B.L. and P.B. Tinker, 1977. Interaction between effects of vesicular-arbuscular mycorrhiza and fertilizer phosphorus on yields of potatoes in the field. Nature 267: 510-511.

Bouwens, H., 1937. Investigations about the mycorrhiza of fruit-trees, especially of quince (Cydonia vulgaris) and of strawberry-plants (Fragaria vesca). Zentralblatt für Bakteriologie II 97: 34-49.

Buwalda, J.G. and K.M. Goh, 1982. Host-fungus competition for carbon as a cause of growth depressions in vesicular-arbuscular mycorrhizal ryegrass. Soil Biology and Biochemistry 14: 103-106.

Carling, D.E., M.F. Brown and R.A. Brown, 1979. Colonization rates and growth responses of soybean plants infected by vesicular-arbuscular mycorrhizal fungi. Canadian Journal of Botany 57: 1769-1772.

Chambers, C.A., S.E. Smith and F.A. Smith, 1980. Effects of ammonium and nitrate ions on mycorrhizal infection, nodulation and growth of Trifolium subterraneum. New Phytologist 85: 47-62.

Chou, L.G. and A.F.S. Schmitthenner, 1974. Effect of Rhi-zobium japonicum and Endogone mosseae on soybean root rot caused by Pythium ultimum and Phytophthora megasperma var. sojae. Plant Disease Reporter 58: 221-225.

Cooper, K.M., 1975. Growth responses to the formation of endotrophic mycorrhizas in Solanum, Leptospermum, and New Zealand ferns. In: Endomycorrhizas. Eds. F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press, London: 391-407.

Cooper, K.M. and P.B. Tinker, 1981. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizas. IV. Effects on environmental variables on movement of phosphorus. New Phytologist 88: 327-339.

Crush, J.R., 1973. The effect of Rhizophagus tenuis mycorrhizas on ryegrass, cocksfoot and sweet vernal. New Phytologist 72: 965-973.

Crush, J.R., 1976. Endomycorrhizas and legume growth in some soils of the Mackenzie Basin, Canterbury, New Zealand. New Zealand Journal of Agricultural Research 19: 473-476.

Daft, M.J. and A.A. El-Giahmi, 1978. Effect of arbuscular mycorrhiza on plant growth. VIII Effects of defoliation and light on selected hosts. New Phytologist 80(2): 365-372.

Daft, M.J. and T.H. Nicolson, 1966. Effect of Endogone mycorrhiza on plant growth. New Phytologist 65: 343-350.

Daft, M.J. and B.D. Okusanya, 1973. Effect of Endogone mycorrhiza on plant growth. VI. Influence of infection on the anatomy and reproductive development in four hosts. New Phytologist 72: 1333-1339.

Davis, R.M., J.A. Menge and D.C. Erwin, 1979. Influence of Glomus fasciculatus and soil phosphorus on Verticillium wilt of cotton. Phytopathology 69: 453-456.

Davis, R.M. and J.A. Menge, 1980. Influence of Glomus fasciculatus and soil phosphorus on Phytophthora root rot of citrus. Phytopathology 70: 447-452.

Downie, D.G., 1957. Corticium solani - an orchid endophyte. Nature, 179: 160.

Dijk, C. van., 1980. The abundance of vesicular arbuscular mycorrhiza in Plantago lanceolata and Plantago coronopus in a natural dune grassland (Westduinen, Goeree). Verhandelingen der Koninklijke Nederlandse Akademie voor Wetenschappen, afdeling Natuurkunde II, 75. Progress Report Institute for Ecological Research 1980: 32-34.

Gerdemann, J.W., 1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infections. Mycologia 47: 619-632.

Gerdemann, J.W., 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annual Review of Phytopathology 6: 397-418.

Gerdemann, J.W., 1975. Vesicular-arbuscular mycorrhizae. In: The development and function of roots. Eds. J.G. Torrey and D.T. Clarkson. Academic Press, London: 575-591.

Gerdemann, J.W. and T.H. Nicolson, 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Transactions of the British Mycological Society 46 (2): 235-244.

Gerdemann, J.W. and J.M. Trappe, 1974. The Endogonaceae in the Pacific Northwest. Mycologia Memoir no. 5: 76 pp.

Gerlagh, M., 1968. Introduction of Ophiobolus graminis into new polders and its decline. Dissertation Agricultural University, Wageningen. 97 pp.

Graham, J.H. and J.A. Menge, 1982. Influence of vesicular-arbuscular mycorrhizae and soil phosphorus on take-all disease of wheat. Phytopathology 72: 95-98.

Graw, D., 1979. The influence of soil pH on the efficiency of vesicular-arbuscular mycorrhiza. New Phytologist 82: 687-695.

Hall, I.R., 1976. Response of Coprosma robusta to different forms of endomycorrhizal inoculum. Transactions of the British Mycological Society 67 (3): 409-411.

Hall, I.R. and B.J. Fish, 1979. A key to the Endogonaceae. Transactions of the British Mycological Society 72 (2): 261-270.

Hall, I.R., R.S. Scott and P.D. Johnstone, 1977. Effect of vesicular-arbuscular mycorrhizas on response of 'Grasslands Huia' and 'Tamar' white clovers to phosphorus. New Zealand Journal of Agricultural Research 20: 349-355.

Hattingh, M.J., L.E. Gray and J.W. Gerdemann, 1973. Uptake and translocation of ^{32}P-labeled phosphate to onion roots by endomycorrhizal fungi. Soil Science 116: 383-387.

Hayman, D.S., 1970. Endogone spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. Transactions of the British Mycological Society 54 (1): 53-63.

Hayman, D.S., 1975. The occurrence of mycorrhiza in crops as affected by soil fertility. In: Endomycorrhizas. Eds. F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press, London: 495-509.

Hayman, D.S., 1978. Endomycorrhizae. In: Interactions between non-pathogenic soil microorganisms and plants. Eds. Y.R. Dommerques and S.V. Krupa. Elsevier, Amsterdam: 401-442.

Hayman, D.S. and B. Mosse, 1972. Plant growth responses to vesicular-arbuscular mycorrhiza. III. Increased uptake of labile P from soil. New Phytologist 71: 41-47.

Hayman, D.S., J.M. Barea and R. Azcon, 1976. Vesicular-arbuscular mycorrhiza in Southern Spain: its distribution in crops growing in soils of different fertility. Phytopathologia Mediterranea 15: 1-6.

Hayman, D.S., A.M. Johnson and I. Ruddlesdin, 1975. The influence of phosphate and crop species on Endogone spores and vesicular-arbuscular mycorrhiza under field conditions. Plant and Soil 43: 489-495.

Heringa, J.W., J. Groenwold and D. Schoonderbeek, 1980. An improved method for the isolation and the quantitative measurement of crop roots. Netherlands Journal of Agricultural Science 28: 127-134.

Jalali, B.L. and K.H. Domsch, 1975. Effect of systemic fungitoxicants on the development of endotrophic mycorrhiza. In: Endomycorrhizas. Eds. F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press, London: 619-626.

Janse, J.M., 1896. Les endophytes radicaux de quelques plantes javanaises. Annales du Jardin Botanique de Buitenzorg, 14: 53-212.

Jensen, A. and I. Jakobsen, 1980. The occurrence of vesicular-arbuscular mycorrhiza in barley and wheat grown in some Danish soils with different fertilizer treatments. Plant and Soil 55: 403-414.

Johnston, A., 1949. Vesicular-arbuscular mycorrhiza in Sea Island cotton and other tropical plants. Tropical Agriculture 26: 118-121.

Joseph, N., 1977. Untersuchungen über den Einfluss des endotrophen Mycorrhizapilzes Glomus mosseae Gerd. & Trappe (Endogone mosseae Nicol. & Gerd.) auf Zea mays L. Dissertation Rheinischen Friedrich-Wilhelms-Universität, Bonn. 105 pp.

Kelley, A.P., 1963. Die Kenntnis der Mykorrhiza vor 1885. In: Mykorrhiza; Internationales Mykorrhizasymposium, Weimar 1960. Eds. W. Rawald und H. Lyr. Fischer Verlag Jena: 1-13.

Khan, A.G., 1974. The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes, and of Endogone spores in adjacent soils. Journal of General Microbiology 81: 7-14.

Khan, A.G., 1975. The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals. II. Effects on wheat growth. Annals of Applied Biology 80: 27-36.

Kruckelmann, H.W., 1973. Die vesikulär-arbuskuläre Mykorrhiza und ihre Beeinflussung in Landwirtschaftlichen Kulturen. Dissertation, Braunschweig, 55 pp.

Kruckelmann, H.W., 1975. Effects of fertilizers, soils, soil tillage and plant species on the frequency of Endogone chlamydospores and mycorrhizal infection in arable soils. In: Endomycorrhizas. Eds. F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press, London: 511-525.

Kuć, J., 1981. Multiple mechanisms, reaction rates and induced resistance in plants. In: Plant disease control. Eds. R.C. Staples and G.H. Thoenniessen. Wiley and Sons, New York: 259-272.

Lambert, D.H., H. Cole jr. and D.E. Baker, 1980. Adaptation of vesicular-arbuscular mycorrhizae to edaphic factors. New Phytologist 85: 513-520.

Lewis, D.H., 1975. Comparative aspects of the carbon nutrition of mycorrhizas. In: Endomycorrhizas. Eds. F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press., London: 119-148.

Maronek, D.M., J.W. Hendrix and J. Kiernan, 1980. Differential growth response to the mycorrhizal fungus Glomus fasciculatus of Southern magnolia and Bar Harbor juniper grown in containers in composted hardwood bark-shale. Journal of the American Society of Horticultural Science 105 (2): 206-208.

Marx, D.H., 1972. Ectomycorrhizae as biological deterrents to pathogenic root infections. Annual Review of Phythopathology 10: 429-454.

Matta, A., 1981. Non phytoalexin host responses in vascular diseases of plants. In: Plant disease control. Eds. R.C. Staples and G.H. Thoenniessen. Wiley and Sons, New York: 179-192.

Mattingly, G.E.G., D.B. Slope and R. Gutteridge, 1980. Annual Report Rothamsted Experimental Station, 1979, part 1: 227-229.

McEwan, G.F., M.W. Johnson and Th.R. Folsom, 1954. A statistical analysis of the performance of the Folsom plankton sample splitter, based upon test observations. Archiv für Meteorologie, Geophysik und Bioklimatologie Serie A 7: 502-527.

McIlveen, W.D. and H. Cole jr., 1976. Spore dispersal of Endogonaceae by worms, ants, wasps and birds. Canadian Journal of Botany 54: 1486-1489.

Mejstrik, V., 1977. Ecology of endomycorrhizae of Trisetum flavescens (L.) P. Beauv. and Alopecurus pratensis L., and the intensity of soil cultivation. Acta Mycologica Vol XIII(1): 179-190.

Menge, J.A., E.L.V. Johnson and V. Minassian, 1979. Effect of heat treatment and three pesticides upon the growth and reproduction of the mycorrhizal fungus Glomus fasciculates. New Phytologist 82: 473-480.

Meyer, F.H., 1973. Distribution of ectomycorrhizae in native and man-made forests. In: Ectomycorrhiza, their ecology and physiology. Eds. G.C. Marks and T.T. Kozlowski. Academic Press., London: 79-105.

Mosse, B., 1953. Fructifications associated with mycorrhizal strawberry roots. Nature, 171: 974.

Mosse, B., 1956. Fructifications of an Endogene species causing endotrophic mycorrhiza in fruit plants. Annals of Botany, N.S. Vol XX, 78: 349-362.

Mosse, B., 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Annual Review of Phytopathology 11: 171-196.

Mosse, B., 1975. Specificity in VA mycorrhizas. In: Endomycorrhizas. Eds. F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press, London: 469-484.

Mosse, B., 1978. Mycorrhiza and plant growth. In: Structure and functioning of plant populations. Eds. A.H.J. Freysen and J.W. Woldendorp, North-Holland Publishing Company, Amsterdam: 269-298.

Mosse, B. and G.D. Bowen, 1968. A key to the recognition of some Endogone spore types. Transactions of the British Mycological Society 51 (3 and 4): 469-483.

Mosse, B., D.S. Hayman and D.J. Arnold, 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labelled with ^{32}P. New Phytologist 72: 803-815.

Nemec, J.A., J.A. Menge, R.G. Platt and E.L.V. Johnson, 1981. Vesicular-arbuscular mycorrhizal fungi associated with citrus in Florida and California and notes on their distribution and ecology. Mycologia 73: 112-127.

Nesheim, O.N. and M.B. Linn, 1969. Deleterious effect of certain fungitoxicants on the formation of mycorrhizae on corn by Endogone fasciculata and on corn root development. Phytopathology 59: 297-300.

Nicolson, T.H., 1967. Vesicular-arbuscular mycorrhiza - a universal plant symbiosis. Sci. Prog. (Oxford) 55: 561-581.

Nicolson, T.H. and N.C. Schenck, 1979. Endogonaceous mycorrhizal endophytes in Florida. Mycologia 71: 178-198.

Nyabyenda, P., 1977. Einfluss der Bodentemperatur und organischer Stoffe in Boden auf die Wirkung der vesikulär-arbuskulären Mycorrhiza. Dissertation, Göttingen, 126 pp.

Ocampo, J.A., 1980. Effect of crop rotations involving host and non-host plants on vesicular-arbuscular mycorrhizal infection of host plants. Plant and Soil 56: 283-291.

Ocampo, J.A. and D.S. Hayman, 1981. Influence of plant interactions on vesicular-arbuscular mycorrhizal infections. II. Crop rotations and residual effects of non-host plants. New Phytologist 87 (2): 333-343.

Ohms, R.E., 1957. A flotation method for collecting spores of a phycomycetous mycorrhizal parasite from soil. Phytopathology 47: 751-752.

Peuss, H., 1958. Untersuchungen zur Oecologie und Bedeutung der Tabakmycorrhiza. Archiv für Mikrobiologie 29: 112-142.

Phillips, J.M. and D.S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society 55: 158-161.

Powell, C.Ll., 1975. Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Uptake of P by onion and clover with different Endogone spore types in ^{32}P labelled soils. New Phytologist 75: 563-566.

Powell, C.Ll., 1976. Development of mycorrhizal infections from Endogone spores and infected root segments. Transactions of the British Mycological Society 66 (3): 439-445.

Powell, C.Ll., 1977. Mycorrhizas in hill-country soils. I. Spore-bearing mycorrhizal fungi in thirty-seven soils. New Zealand Journal of Agricultural Research 20: 53-57.

Ratnayake, M., R.T. Leonard and J.A. Menge, 1978. Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. New Phytologist 81(3): 543-552.

Reeves, F.B., D. Wagner, T. Moorman and J. Kiel, 1979. The role of endomycorrhizae in revegetation practices in the semi-arid West. I. A comparison of incidence of mycorrhizae in severely disturbed vs. natural ecosystems. American Journal of Botany 66: 6-13.

Ross, J.P., 1972. Influence of Endogone mycorrhiza on Phytophthora rot of soybean. Phytopathology 62: 896-897.

Rothwell, F.M. and C. Holt, 1978. Vesicular-arbuscular mycorrhizae established with Glomus fasciculatus spores isolated from the feces of cricetine mice. Forest Service Research Note NE-259, 4 pp. Forest Service USDA.

Sanders, F.E., 1975. Effect of foliar-applied phosphate on the mycorrhizal infections of onion roots. In: Endomycorrhizas. Eds. F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press, London: 261-276.

Sanders, F.E. and P.B. Tinker, 1971. Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature, London 233: 278-279.

Schenck, N.C. and M.K. Kellam, 1978. The influence of vesicular-arbuscular mycorrhizae on disease development. Bulletin 798, Agricultural Experiment Stations, University of Florida, 16 pp.

Schenck, N.C. and R.A. Kinloch, 1980. Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. Mycologia 72: 445-456.

Schenck, N.C. and G.S. Smith, 1982. Additional new and unreported species of mycorrhizal fungi (Endogonaceae) from Florida. Mycologia 74 (1): 77-92.

Schlicht, A., 1889. Beitrag zur Kenntnis der Verbreitung und der Bedeutung der Mykorhizen. Landwirthschaftliche Jahrbücher 18: 477-506.

Schoknecht, J.D. and M.J. Hattingh, 1976. X-ray micro-analysis of elements in cells of VA-mycorrhizal and nonmycorrhizal onions. Mycologia 68: 296-303.

Schönbeck, F., 1978. Endomycorrhiza in relation to plant diseases. In: Soil borne plant diseases. Eds. B. Schippers and W. Gams. Academic Press, London: 271-280.

Schönbeck, F. and H.W. Dehne, 1977. Damage to mycorrhizal and nonmycorrhizal cotton seedlings by Thielaviopsis basicola. Plant Disease Reporter 61: 266-267.

Schönbeck, F. and H.W. Dehne, 1979. Untersuchungen zum Einfluss der endotrophen Mycorrhiza auf Pflanzenkrankheiten. 4. Pilzliche Sprossparasiten, Olpidium brassicae, TMV. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 86 (2): 103-112.

Slankis, V., 1974. Soil factors influencing formation of mycorrhizae. Annual Review of Phytopathology 12: 437-457.

Smith, T.F., 1980. The effect of season and crop rotation on the abundance of spores of vesicular-arbuscular (V-A) mycorrhizal endophytes. Plant and Soil 57: 475-479.

Smith, S.S.E., 1980. Mycorrhizas of autotrophic higher plants. Biological Reviews 55: 475-510.

Smith, F.A. and S.E. Smith, 1981. Mycorrhizal infection and growth of Trifolium subterraneum: comparison of natural and artificial inocula. New Phytologist 88: 311-325.

Smith, G.W. and H.D. Skipper, 1979. Comparison of methods to extract spores of vesicular-arbuscular mycorrhizal fungi. Soil Science Society of America Journal 43: 722-725.

Sparling, G.P. and P.B. Tinker, 1978. Mycorrhizal infection in Pennine grassland. I. Levels of infection in the field. Journal of Applied Ecology 15: 943-950.

Stahl, E., 1900. Der Sinn der Mycorhizenbildung. Jahrbücher für wissenschaftliche Botanik, 34: 539-668.

Stribley, D.P., P.B. Tinker and J.H. Rayner, 1980. Relation of internal phosphorus concentration and plant weight in plants infected by vesicular-arbuscular mycorrhizas. New Phytologist 86: 261-266.

Strzemska, J., 1975. Mycorrhiza in farm crops grown in monoculture. In: Endomycorrhizas. Eds. F.E. Sanders, B. Mosse and P.B. Tinker. Academic Press, London: 527-535.

Tesarová, M., 1972. Effect of phosphorus on the rate of cellulose decomposition in soil. In: Ecosystem study on Grassland Biome in Czechoslovakia. Czechosl. IBP/PT-PP Report no. 2, Brno.

Tolle, R., 1958. Untersuchungen über die Pseudomycorrhiza von Gramineen. Archiv für Mikrobiologie 30: 285-303.

Tommerup, I.C. and G.G. Briggs, 1981. Influence of agricultural chemicals on germination of vesicular-arbuscular endophyte spores. Transaction of the British Mycological Society 76 (2): 326-328.

Trappe, J.M. and C. Maser, 1976. Germination of spores of Glomus macrocarpus (Endogonaceae) after passage through a rodent digestive tract. Mycologia 68: 433-436.

Trappe, J.M., E.A. Stahly, N.R. Benson and D.M. Duffe, 1973. Mycorrhizal deficiency of apple trees in high arsenic soils. Hortscience 8 (1): 52-53.

Treub, M., 1885. Onderzoekingen over Sereh-ziek suikerriet gedaan in 's Lands Plantentuin te Buitenzorg. Mededeelingen uit 's Lands Plantentuin II: 1-39.

Trudgill, D.L., K. Evans and G. Faulkner, 1972. A fluidising column for extracting nematodes from soil. Nematologia 18: 469-475.

Vries, G.A. de., 1971. De fungi van Nederland. 3. Hypogaea. Wetenschappelijke mededelingen van de Koninklijke Nederlandse Natuurhistorische Vereniging 88: 1-62.

Zambolin, L. and N.C. Schenck, 1980a. Effect of root rot fungi and VA-mycorrhiza on the establishment of Rhizobium japonicum on nodulated and non-nodulated "Hardee" soybeans. Abstract 494 APS-CPS Annual Meeting Minneapolis.

Zambolin, L. and N.C. Schenck, 1980b. Interactions between a VA-mycorrhiza and root infecting fungi on soybean. Abstract 495 of the APS-CPS Annual Meeting Minneapolis.

CURRICULUM VITAE

Matheus Adriaan Ruissen werd op 28 november 1949 te Melissant geboren. Na het behalen van het diploma hbs-B aan het Corderius Lyceum te Amersfoort en een korte maatschappelijke loopbaan, begon hij in 1970 met de studie aan de Landbouwhogeschool te Wageningen. In maart 1977 legde hij het doctoraalexamen af in de studierichting Planteziektenkunde met als hoofdvak Fytopathologie (verzwaard) en de bijvakken Microbiologie en Erfelijkheidsleer.
Gedurende het studiejaar 1977-1978 was hij als leraar planteziektenkunde/biologie verbonden aan de Rijks Agrarische Scholengemeenschap te Boskoop. Van september 1978 tot november 1981 was hij als wetenschappelijk assistent werkzaam bij de vakgroep Fytopathologie van de Landbouwhogeschool en verwerkte daarna, de in die periode verzamelde gegevens tot dit proefschrift. Vanaf 1 september 1982 is hij, als wetenschappelijk medewerker in tijdelijke dienst, werkzaam bij de vakgroep Fytopathologie van de Landbouwhogeschool.

omslag ontwerp : frederik v. planta