OCCURRENCE AND SIGNIFICANCE OF VESICULAR-ARBUSCULAR MYCORRHIZAE IN SASKATCHEWAN SOILS AND FIELD CROPS

A Thesis
Submitted to the Faculty of Graduate Studies and Research
in Partial Fulfillment of the Requirements
for the Degree of

Doctor of Philosophy

in the

Department of Soil Science
University of Saskatchewan

by

Narayan Chandra Talukdar
Saskatoon, Saskatchewan, Canada
January, 1993

Copyright © N. C. Talukdar 1992
The author has agreed that the Library, University of Saskatchewan, may make this thesis freely available for inspection. Moreover, the author has agreed that permission for extensive copying of this thesis for scholarly purposes may be granted by the professor or professors who supervised the thesis work recorded herein or, in their absence, by the Head of the Department or the Dean of the College in which the thesis work was done. It is hereby understood that due recognition will be given to the author of this thesis and to the University of Saskatchewan in any use of the thesis. Copying or publication or any other use of the thesis for financial gain without approval by the University of Saskatchewan and the author's written permission is prohibited.

Request for permission to copy or to make any other use of the material in this thesis in whole or in part should be addressed to:

Head of the Department of Soil Science
University of Saskatchewan
SASKATOON, Saskatchewan
Canada S7N 0W0
ABSTRACT

Vesicular-arbuscular mycorrhizal (VAM) fungi form an association with most crop roots. Knowledge on the occurrence of VAM in soils and their symbiosis with crops is important to understand the impact of VAM on crops in agroecosystems. This information may be used to manage VAM so as to maximize their contribution to plant growth and development. My research assessed the occurrence and significance of VAM in Saskatchewan soils and field crops. This was accomplished through a series of field and growth chamber experiments designed to:

(i) study the occurrence of VAM in wheat and lentil and to isolate different VAM from wheat field soils.

(ii) assess the effect of crop rotations on VAM infection in field grown crops.

(iii) develop a method to propagate monospecific cultures of VAM strains isolated from Saskatchewan soils.

(iv) study the effect of selected VAM inocula on growth of wheat and lentil.

Soil and root samples collected from wheat and lentil fields at 11 sites during 1988 and 1989 were analyzed for spore numbers, level of VAM infection, and VAM species. The number of VAM spores detected in field soils ranged from 78-272 per 100 g soil in the 1988 study and 410-2113 per 100 g soil in the 1989 study. Vesicular-arbuscular mycorrhizae infected wheat and lentil at all the field study sites, but levels of infection in the two crops varied from site to site and the differences were more pronounced in wheat than in lentil. Differences in VAM infection in wheat at different sites were not related to the moisture and temperature gradient of the four soil zones or soil properties. Generally, lentil exhibited both a higher percentage of VAM infected roots, and contained more...
Glomus mosseae and *G. versiforme*, and the level of spore production in soil was: *G. geosporum* > *G. versiforme* > *G. mosseae*. The ability of *G. geosporum* to produce a large number of spores reflected either an inherent ability, or a more favorable response to maize as host crop or the conditions in the growth chamber.

The ability of *G. geosporum*, *G. mosseae* and *G. versiforme* to infect wheat and lentil and the response of the two crops to VAM infection were assessed in sterile soil under growth chamber conditions. Seedlings were inoculated with 100 VAM spores of a species, or an equal mixture of two species. The two crops responded differently depending on the VAM species. In the case of lentil, *G. geosporum* was more effective than *G. versiforme* and *G. mosseae*, and increased the shoot dry weight (37%) and grain yield (56%) compared to the uninoculated control. There was a significant positive correlation between the percentage of VAM infected roots and shoot dry weight of lentil at 31 and 56 days after planting, but not at harvest. Inoculation also increased the P content of lentil shoots, following a trend similar to that for infection. *Glomus geosporum* increased the shoot weight, grain yield (12%) and the shoot and grain P content of wheat. Although *G. geosporum* and *G. mosseae* both produced similar levels of infection in wheat, the only response of wheat to *G. mosseae* was an increase in plant height at harvest. The efficacy of *G. geosporum* on both crops appeared to be related to its ability to produce a higher percentage of arbuscular infection compared to *G. mosseae*.

Co-inoculation of seedlings with *G. geosporum* and *G. mosseae* resulted in competition between the two VAM. This was evident from a comparison of shoot dry weight and spore production on the two crops inoculated with either *G. geosporum* or *G. geosporum* plus *G. mosseae*. *Glomus mosseae* reduced the efficacy of *G. geosporum* by 16% when co-inoculated on lentil, but had no effect when the host was wheat. Based on spore production it was found that *G. geosporum* was more competitive than *G. mosseae* when co-inoculated to lentil and wheat. *Glomus geosporum* produced ca. 2000 and 500
spores per 100 g substrate, respectively in lentil and wheat soil, which was approximately 2 to 3 fold more spores than produced by *G. mosseae*. When co-inoculated, there was a 15 to 19% reduction in spore production by *G. geosporum* versus a 50-70% decrease in spore production by *G. mosseae*. These results indicated that these VAM species exhibit a host specificity in terms of their ability to infect and increase the growth and yield of lentil and wheat. *Glomus geosporum* was more competitive and effective than *G. mosseae* or *G. versiforme* as reflected by its positive impact on growth of lentil and wheat.

The difference in the ability of wheat and lentil to support spore production by *G. geosporum*, *G. mosseae* and *G. versiforme* isolates from Saskatchewan soils under growth chamber conditions suggested that these crops may have a similar effect in agricultural fields. Thus, depending upon cropping practices the dynamics of VAM spore populations in soil may change. In addition, production of canola or summerfallow influenced the population dynamics of indigenous VAM (i.e, spore numbers), resulting in reduced infection of subsequent wheat and barley. The number of spores recovered from the canola and summerfallow field soils was 11 to 39% and 18 to 54% lower, respectively than that recovered from the adjacent wheat field soils. The low number of spores resulted in low levels of infection in the subsequent wheat and barley crops grown in these canola and summerfallow fields. Thus my results suggest that cropping practices influenced the population dynamics and activity of indigenous VAM and their potential benefits to field grown crops in Saskatchewan.
DEDICATION

This thesis is dedicated to my mother.
ACKNOWLEDGEMENTS

I wish to express my heartfelt gratitude to Dr. J. J. Germida for his supervision, encouragement during this study and invaluable suggestions during the preparation of the manuscript. Gratitude is also extended to the members of the supervisory committee, Drs. Chris van Kessel, Douglas R. Waterer, Louise M. Nelson and Darwin Anderson for their suggestions during the course of this research and the critical review of the manuscript.

I am truly grateful to Canadian Commonwealth Scholarship and fellowship plan for selecting me for an award to pursue this study.

I thank my colleagues and friends who gave me help and friendship during the last five years: Renato de Freitas, Tabo Mubyana, V. V. S. R. Gupta, Fran Walley, Richard Farrell, Sheilla Skinnider, Sandra Hofer, Adele Lukey, Tracy Stewart, Darren Hill, Liset Johnny. I would also like to thank Jackie Moir for her help in carrying out nutrient analysis of the plant materials.

The friendship and assistance of fellow graduate students, staff and faculty members of Saskatchewan Institute of Pedology is greatly appreciated. Finally, I would like to thank my brother Bani for his help and support during the last year.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2 LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>2.1 A historical perspective of vesicular-arbuscular mycorrhizae (VAM)</td>
<td>5</td>
</tr>
<tr>
<td>2.2 VAM structures and their function in the symbiosis</td>
<td>6</td>
</tr>
<tr>
<td>2.3 Taxonomy of VAM</td>
<td>9</td>
</tr>
<tr>
<td>2.4 Identification of VAM</td>
<td>14</td>
</tr>
<tr>
<td>2.4.1 Criteria for VAM identification</td>
<td>14</td>
</tr>
<tr>
<td>2.5 Occurrence and distribution of VAM</td>
<td>20</td>
</tr>
<tr>
<td>2.5.1 Distribution of VAM in the plant kingdom</td>
<td>20</td>
</tr>
<tr>
<td>2.5.2 Occurrence of VAM in major plant habitats</td>
<td>21</td>
</tr>
<tr>
<td>2.5.3 Significance of VAM infection of plant communities in natural habitats</td>
<td>23</td>
</tr>
<tr>
<td>2.5.4 Effects of agriculture on the VAM of natural ecosystems</td>
<td>24</td>
</tr>
<tr>
<td>2.5.5 Occurrence of VAM in agricultural soils and crops</td>
<td>24</td>
</tr>
<tr>
<td>2.5.5.1 VAM infection and spore numbers in cereals and legumes</td>
<td>24</td>
</tr>
<tr>
<td>2.5.5.2 Influence of soil properties on mycorrhizal status of field grown crops</td>
<td>28</td>
</tr>
<tr>
<td>2.5.6 Distribution of VAM species</td>
<td>28</td>
</tr>
<tr>
<td>2.5.6.1 Techniques used to study the distribution of VAM species</td>
<td>29</td>
</tr>
<tr>
<td>2.5.6.2 Factors affecting distribution of VAM species</td>
<td>30</td>
</tr>
<tr>
<td>2.5.7 Influence of agricultural practices on VAM spore numbers in soils and VAM infection in field grown crops</td>
<td>31</td>
</tr>
<tr>
<td>2.6 Production of inocula of VAM isolates</td>
<td>37</td>
</tr>
<tr>
<td>2.6.1 Factors affecting production of VAM inocula</td>
<td>39</td>
</tr>
<tr>
<td>2.6.2 Factors affecting the viability of VAM inocula</td>
<td>42</td>
</tr>
<tr>
<td>Chapter</td>
<td>Title</td>
</tr>
<tr>
<td>---------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>2.7</td>
<td>Plant growth response to VAM inocula</td>
</tr>
<tr>
<td>2.7.1</td>
<td>Effect of VAM inoculation on growth and yield of crops in sterilized soil</td>
</tr>
<tr>
<td>2.7.2</td>
<td>Effect of VAM inoculation on growth and yield of crops in natural soil containing indigenous VAM</td>
</tr>
<tr>
<td>2.7.3</td>
<td>Effect of VAM inoculation on plant root growth</td>
</tr>
<tr>
<td>2.7.4</td>
<td>Efficacy of VAM isolates to promote plant growth</td>
</tr>
<tr>
<td>2.7.4.1</td>
<td>Reasons for difference in efficacy between VAM species</td>
</tr>
<tr>
<td>2.7.4.2</td>
<td>Performance of VAM under varying host-environment conditions</td>
</tr>
<tr>
<td>2.7.5</td>
<td>Growth depression of host crops by VAM isolates</td>
</tr>
<tr>
<td>2.7.6</td>
<td>Mycorrhizal dependency of host crops</td>
</tr>
<tr>
<td>2.7.7</td>
<td>Interaction between VAM isolates</td>
</tr>
<tr>
<td>2.7.8</td>
<td>Interaction between host genotypes and VAM</td>
</tr>
<tr>
<td>2.8</td>
<td>General conclusions</td>
</tr>
<tr>
<td>3</td>
<td>EXPERIMENTAL</td>
</tr>
<tr>
<td>3.1</td>
<td>Occurrence and isolation of vesicular-arbuscular mycorrhizae in cropped field soils of Saskatchewan</td>
</tr>
<tr>
<td>3.1.1</td>
<td>Introduction</td>
</tr>
<tr>
<td>3.1.2</td>
<td>Materials and Methods</td>
</tr>
<tr>
<td>3.1.2.1</td>
<td>Field study sites</td>
</tr>
<tr>
<td>3.1.2.2</td>
<td>Determination of VAM spore numbers</td>
</tr>
<tr>
<td>3.1.2.3</td>
<td>VAM infection of wheat and lentil</td>
</tr>
<tr>
<td>3.1.2.4</td>
<td>Isolation and identification of VAM</td>
</tr>
<tr>
<td>3.1.2.5</td>
<td>Occurrence of VAM species in different soils</td>
</tr>
<tr>
<td>3.1.3</td>
<td>Results</td>
</tr>
<tr>
<td>3.1.3.1</td>
<td>VAM spore numbers</td>
</tr>
<tr>
<td>3.1.3.2</td>
<td>VAM infection in wheat and lentil</td>
</tr>
<tr>
<td>3.1.3.3</td>
<td>Isolation and identification of VAM</td>
</tr>
<tr>
<td>3.1.3.4</td>
<td>Distribution of VAM species in soils</td>
</tr>
<tr>
<td>3.1.4</td>
<td>Discussion</td>
</tr>
</tbody>
</table>

viii
### Chapter 3.2 Effects of crop rotation and soil phosphorus levels on vesicular-arbuscular mycorrhizae in field grown crops in Saskatchewan

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.1 Introduction</td>
<td>83</td>
</tr>
<tr>
<td>3.2.2 Materials and Methods</td>
<td>84</td>
</tr>
<tr>
<td>3.2.2.1 Effect of canola or summerfallow on VAM infection of wheat or barley crops</td>
<td>84</td>
</tr>
<tr>
<td>3.2.2.2 Relationship between available P and VAM infection</td>
<td>86</td>
</tr>
<tr>
<td>3.2.3 Results</td>
<td>89</td>
</tr>
<tr>
<td>3.2.3.1 Effect of canola or summerfallow on VAM infection of wheat or barley crops</td>
<td>89</td>
</tr>
<tr>
<td>3.2.3.2 Relationship between available P and VAM infection</td>
<td>93</td>
</tr>
<tr>
<td>3.2.4 Discussion</td>
<td>93</td>
</tr>
</tbody>
</table>

### Chapter 3.3 Propagation and storage of propagules of vesicular-arbuscular mycorrhizae isolated from Saskatchewan agricultural soils

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.1 Introduction</td>
<td>97</td>
</tr>
<tr>
<td>3.3.2 Materials and Methods</td>
<td>98</td>
</tr>
<tr>
<td>3.3.2.1 Optimization of spore production by <em>Glomus geosporum</em></td>
<td>98</td>
</tr>
<tr>
<td>3.3.2.1.1 Inoculation of host crops and plant growth conditions</td>
<td>100</td>
</tr>
<tr>
<td>3.3.2.1.2 Growth parameters, VAM infection and spore production</td>
<td>100</td>
</tr>
<tr>
<td>3.3.2.1.3 Nutrient content</td>
<td>101</td>
</tr>
<tr>
<td>3.3.2.2 Production of monospecific cultures of <em>G. geosporum, G. mosseae</em> and <em>G. versiforme</em></td>
<td>101</td>
</tr>
<tr>
<td>3.3.2.3 Effects of temperature and osmotic stress on <em>G. mosseae</em> spores</td>
<td>102</td>
</tr>
<tr>
<td>3.3.2.3.1 Inoculation and growth conditions of plants</td>
<td>104</td>
</tr>
<tr>
<td>3.3.2.3.2 Estimation of VAM infection and plant parameters</td>
<td>104</td>
</tr>
<tr>
<td>3.3.2.4 Statistical analysis</td>
<td>104</td>
</tr>
<tr>
<td>Chapter</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>3.3.3</td>
<td>105</td>
</tr>
<tr>
<td>3.3.3.1</td>
<td>105</td>
</tr>
<tr>
<td>3.3.3.2</td>
<td>108</td>
</tr>
<tr>
<td>3.3.3.3</td>
<td>112</td>
</tr>
<tr>
<td>3.3.4</td>
<td>112</td>
</tr>
</tbody>
</table>

3.4 Growth of lentil and wheat inoculated with *Glomus geosporum, Glomus mosseae* and *Glomus versiforme*

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4.1</td>
<td>120</td>
</tr>
<tr>
<td>3.4.2</td>
<td>120</td>
</tr>
<tr>
<td>3.4.2.1</td>
<td>121</td>
</tr>
<tr>
<td>3.4.2.2</td>
<td>121</td>
</tr>
<tr>
<td>3.4.2.2.1</td>
<td>122</td>
</tr>
<tr>
<td>3.4.2.2.2</td>
<td>122</td>
</tr>
<tr>
<td>3.4.2.2.3</td>
<td>124</td>
</tr>
<tr>
<td>3.4.2.2.4</td>
<td>124</td>
</tr>
<tr>
<td>3.4.2.3</td>
<td>125</td>
</tr>
<tr>
<td>3.4.2.4</td>
<td>125</td>
</tr>
<tr>
<td>3.4.3</td>
<td>126</td>
</tr>
<tr>
<td>3.4.3.1</td>
<td>126</td>
</tr>
<tr>
<td>3.4.3.1.1</td>
<td>126</td>
</tr>
<tr>
<td>3.4.3.1.2</td>
<td>126</td>
</tr>
<tr>
<td>3.4.3.1.3</td>
<td>130</td>
</tr>
<tr>
<td>3.4.3.2</td>
<td>130</td>
</tr>
<tr>
<td>3.4.3.2.1</td>
<td>130</td>
</tr>
<tr>
<td>3.4.3.2.2</td>
<td>130</td>
</tr>
<tr>
<td>3.4.3.2.3</td>
<td>135</td>
</tr>
<tr>
<td>3.4.3.2.4</td>
<td>135</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>2.5.1</td>
<td>The effect of pH on the distribution of spores of VAM fungi in the Merredin area of Western Australia (Adapted from Porter et al. 1987)</td>
</tr>
<tr>
<td>2.5.2</td>
<td>The effect of applying phosphate on colonization of barley roots by VAM fungi at five field sites in the Netherlands (Adapted from Jensen and Jacobsen 1980)</td>
</tr>
<tr>
<td>2.7.1</td>
<td>Growth of different host crops inoculated with VAM and grown in sterilized soils of different pH and with different levels of plant available P.</td>
</tr>
<tr>
<td>2.7.2</td>
<td>Competition between VAM species in terms of host growth, VAM infection and spore production in the root region of host plants inoculated with a single VAM or a mixture of VAM species</td>
</tr>
<tr>
<td>2.7.3</td>
<td>Differential response of plant genotypes to VAM inoculation as determined by root infection and shoot growth</td>
</tr>
<tr>
<td>3.1.2.1</td>
<td>Soil and crop characteristics of VAM survey sites</td>
</tr>
<tr>
<td>3.1.3.1</td>
<td>Vesicular-arbuscular mycorrhizal spore numbers in Saskatchewan field soils at seeding of 1988 wheat crop</td>
</tr>
<tr>
<td>3.1.3.2</td>
<td>Available soil moisture at seeding and precipitation during the 1988 growing season at Saskatchewan field sites</td>
</tr>
<tr>
<td>3.1.3.3</td>
<td>Vesicular-arbuscular mycorrhizal spores isolated from the soil:sand mix used to grow maize inoculated with VAM spore mixtures indigenous to field soils.</td>
</tr>
<tr>
<td>3.2.2.1</td>
<td>Soil characteristics of wheat and barley field sites under different rotations</td>
</tr>
<tr>
<td>3.2.3.1</td>
<td>Number of VAM spores in canola, summerfallow and wheat field soils</td>
</tr>
<tr>
<td>Table</td>
<td>Description</td>
</tr>
<tr>
<td>-------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>3.2.3.2</td>
<td>Number of VAM spores in wheat and barley field soils previously cropped to canola or wheat, or kept in summerfallow</td>
</tr>
<tr>
<td>3.3.2.1</td>
<td>Characteristics of soil:sand (1:1) mix before and after sterilization</td>
</tr>
<tr>
<td>3.3.3.1</td>
<td>Production of <em>Glomus geosporum</em> spores as influenced by host crop and soil volume</td>
</tr>
<tr>
<td>3.3.3.2</td>
<td>Spore production and infection level of <em>Glomus geosporum</em>, <em>Glomus mosseae</em> and <em>Glomus versiforme</em> grown on maize</td>
</tr>
<tr>
<td>3.3.3.3</td>
<td>Effect of temperature and osmotic stress on the viability and (or) infectivity of <em>Glomus mosseae</em> spores as determined by percent VAM infection, spore production and growth of wheat and sorghum-sudangrass hybrid</td>
</tr>
<tr>
<td>3.4.3.1</td>
<td>Growth and yield of lentil inoculated with <em>Glomus geosporum</em>, <em>Glomus mosseae</em> or <em>Glomus versiforme</em> spores and grown in a growth chamber</td>
</tr>
<tr>
<td>3.4.3.2</td>
<td>Phosphorus content of shoots and grain of lentil inoculated with <em>Glomus geosporum</em>, <em>Glomus mosseae</em> or <em>Glomus versiforme</em> and grown in a growth chamber</td>
</tr>
<tr>
<td>3.4.3.3</td>
<td>Growth and yield of wheat inoculated with <em>Glomus geosporum</em> or <em>Glomus mosseae</em> spores and grown in a growth chamber</td>
</tr>
<tr>
<td>3.4.3.4</td>
<td>Phosphorus content of shoots and grain of wheat inoculated with <em>Glomus geosporum</em> or <em>Glomus mosseae</em> and grown in a growth chamber</td>
</tr>
<tr>
<td>3.4.3.5</td>
<td>Vesicular-arbuscular mycorrhizal spores recovered at harvest from soil:sand mix used to grow wheat and lentil under growth chamber conditions</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Photomicrographs of infected root segments showing typical structures of vesicular-arbuscular mycorrhizae. (A) Vesicles (ve), arbuscules (ar), internal and extramatrical hyphae (h) and external chlamydospores (sp), and the root segments infected with (B) arbuscules, (C) vesicles, (D) internal hyphae and (E) extramatrical hyphal network (h).</td>
<td>7</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Classification of vesicular-arbuscular mycorrhizae in the family Endogonaceae (Adapted from Gerdemann and Trappe 1974). The bold letters represent different taxa of fungi that form vesicular-arbuscular mycorrhizae.</td>
<td>11</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Revised classification of vesicular-arbuscular mycorrhizal fungi in the class Zygomycetes (Adapted from Schenck and Perez 1988; Morton and Benny 1990).</td>
<td>13</td>
</tr>
<tr>
<td>2.4.1</td>
<td>Groups of spores belonging to: (A) Glomus geosporum, (B) Glomus fasciculatum, (C) Glomus mossaeae and (D) Glomus versiforme.</td>
<td>16</td>
</tr>
<tr>
<td>2.4.2</td>
<td>Murograph of wall type, number, group and position in a VA mycorrhizal fungus spore. (O) = ornamental surface, * = difficult to see. Lines connecting two walls depict a cementing layer. Walls numbered consecutively from the outer to inner surface. 1 = expanding wall, 2 = evanescent, 3 = laminated, 4-7 = unit, 8 = membranous, 9 = coriaceous, 10 = amorphous walls. Each wall group is bracketed in parentheses with consecutive letters from outer to inner surface (Adapted from Morton 1988).</td>
<td>19</td>
</tr>
<tr>
<td>2.5.1</td>
<td>The development of mycorrhizae in (A) Bean (Phaseolus vulgaris L. cv. Topcrop) (Adapted from Sutton 1973), (B) Field pea (Pisum sativum L. cv. Bondi) (Adapted from Jakobsen and Nielsen 1983), and (C) winter wheat (Triticum aestivum L. cv. Avalon) grown at G-1 site (Adapted from Dodd and Jeffries 1986).</td>
<td>26</td>
</tr>
<tr>
<td>3.1.2.1</td>
<td>Location of VAM survey sites in Saskatchewan. Note: DB=Dark Brown, BL=Black, GR=Gray.</td>
<td>63</td>
</tr>
</tbody>
</table>
3.1.2.2 Mixture of VAM spores collected from maize rhizosphere (A) before and (B) after sucrose density gradient (20% and 60%) centrifugation.

3.1.3.1 Vesicular-arbuscular mycorrhizal infection at different growth stages of wheat (●) and lentil (□) grown during 1988 and 1989 in the Brown and Dark Brown soil zones. Infection values are means of 3 subsamples ± S.D.

3.1.3.2 Vesicular-arbuscular mycorrhizal infection at different growth stages of wheat (●) and lentil (□) grown during 1988 and 1989 in the Black and Gray soil zones. Infection values are means of 3 subsamples ± S.D.

3.1.3.3 Typical root segments of 50 day old field grown (Outlook site) lentil (A) and (B) containing abundant arbuscules (ar) and vesicles (ve) and wheat (C and D) containing few arbuscules and vesicles. Bar markers = 180 μm.

3.1.3.4 Different types of vesicle structures (arrows) observed in wheat roots collected at different field sites: (A and B) Detected at Outlook, Semans and Medstead. (C) Detected at Semans and Medstead. (D) Detected at Semans only. (E) Detected at Outlook and Medstead. (F) Detected at Medstead only. Bar markers = 63 μm.

3.1.3.5 Spores of vesicular-arbuscular mycorrhizal fungi indigenous to Saskatchewan field soils. Representative spores were mounted in polyvinyl alcohol-lactic acid-glycerol and photographed using differential interference contrast microscopy, except (C) which was unmounted and photographed using stereomicroscopy. (A) *Acaulospora denticulata* Sieverding and Toro; arrows show four distinct spore walls. (B) Close-up of *Acaulospora denticulata* spore showing characteristic polygon segments (arrow) of the outer wall. (C) *Gigaspora decipiens* Hall and Abbott; arrow shows bulbous suspensor. (D) Squashed *Gigaspora decipiens* spores showing distinct spore walls (arrows). (E) *Glomus fasciculatum* Walker and Koske; loose cluster of spores. (F) *Glomus fasciculatum* spores showing attachment (arrow) of hyphae to spore wall. (G) Close-up of *Glomus fasciculatum* spore showing ingrowths (arrows).
from wall. (H) *Glomus geosporum* Nicolson and Gerdemann; arrow shows thick hyphal wall at the point of attachment to the spore. (I) Squashed *Glomus geosporum* spore showing spore wall details (arrows). (J) Intact spores of *Glomus macrocarpum* (Tul. and Tul.) Berch and Fortin. (K) Squashed *Glomus macrocarpum* spore showing spore walls (arrows). (L) *Glomus mosseae* (Nicolson and Gerdemann) Gerdemann and Trappe; arrow shows characteristic funnel shape of hyphal attachment. (M) Close-up of squashed *Glomus mosseae* spore showing spore walls (arrows). (N) A portion of a *Glomus versiforme* (Karsten) Berch sporocarp. (O) Individual *Glomus versiforme* spore showing characteristic spore walls (arrows).

All bar markers = 65 µm. .............................................. 77

3.2.2.1 A typical field with three adjacent crop rotations. (Aberdeen site). .......................................................... 87

3.2.2.2 Typical catenary sequence sampled at wheat and lentil field sites. ................................................................. 88

3.2.3.1 Effect of previous year crop on VAM infection in wheat and barley grown in the subsequent year; wheat (●), canola (○) and summerfallow (□). Infection values are means of three subsamples ± S.D. ......................... 92

3.2.3.2 Relationship between available P and % VAM infected root length wheat and lentil at 20 (A), 50 (B) and 70 (C) days after emergence at different study sites. .......................... 94

3.3.2.1 Pot system for production of monospecific VAM cultures. ....... 103

3.3.3.1 Nutrient deficiency symptoms of 56 day old maize plants grown in 2 kg soil:sand mix. Note yellow purple color of leaves. ................................................................. 107

3.3.3.2 Spores of (A) *Glomus geosporum*, (B) *Glomus mosseae* and (C) *Glomus versiforme* produced in monospecific cultures on a maize host. Bar marker = 300 µm. .......................... 110
<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3.3.3</td>
<td>Size distribution of (A) <em>Glomus geosporum</em>, (B) <em>Glomus mosseae</em> and (C) <em>Glomus versiforme</em> spores in their monospecific cultures. Spores from a 25-g sample were suspended in tap water and passed through sieves of decreasing pore size. Spores retained on each sieve were counted under a stereomicroscope (47×). Each bar represents the average spore number of three replicate samples .............................................. 111</td>
</tr>
<tr>
<td>3.3.3.4</td>
<td>Response of wheat plants to inoculation with <em>Glomus mosseae</em> spores stored at 7°C ................................................................. 114</td>
</tr>
<tr>
<td>3.4.2.1</td>
<td>Funnel technique used to inoculate seedlings of wheat and lentil ........................................................................................................... 123</td>
</tr>
<tr>
<td>3.4.3.1</td>
<td>Effect of inoculation with <em>Glomus geosporum</em> (△), <em>Glomus mosseae</em> (○), <em>Glomus geosporum</em> + <em>Glomus mosseae</em> (●) and <em>Glomus versiforme</em> (□) on the percentage of (A) total VAM infection and (B) arbuscular infection in lentil roots at 3 harvests. Vertical lines represent least significant differences between treatment means (P = 0.05) ................................................................ 128</td>
</tr>
<tr>
<td>3.4.3.2</td>
<td>Relationship between shoot dry weight and percentage of VAM infection in lentil roots at (A) 31 and (B) 56 days after planting. **, statistically significant at P = 0.01 .................................................................................................................. 129</td>
</tr>
<tr>
<td>3.4.3.3</td>
<td>Effect of <em>Glomus geosporum</em> and <em>Glomus mosseae</em> on the growth of wheat at 48 days after planting. Note early panicle emergence (arrows) in <em>Glomus geosporum</em>-inoculated plants ............................................................ 132</td>
</tr>
<tr>
<td>3.4.3.4</td>
<td>Effect of <em>Glomus geosporum</em> and <em>Glomus mosseae</em> on the growth of wheat at 83 days after planting. Note tiller numbers (white arrow) in <em>Glomus geosporum</em>-inoculated plants and height of <em>Glomus mosseae</em>-inoculated plants ........................................................................................................ 133</td>
</tr>
<tr>
<td>3.4.3.5</td>
<td>Effect of inoculation with <em>Glomus geosporum</em> (△), <em>Glomus mosseae</em> (○) and <em>Glomus geosporum</em> + <em>Glomus mosseae</em> (●) on the percentage of (A) total VAM infection and (B) arbuscular infection in wheat roots at 3 harvests. Vertical lines represent least significant differences (P = 0.05) between treatment means ........................................................................................................ 136</td>
</tr>
</tbody>
</table>
3.4.3.6 Photomicrographs of 56 day old lentil root showing infection morphology of *Glomus geosporum*, *Glomus mosseae* and *Glomus versiforme* inside wheat roots. (A) Abundant arbuscule (ar) production by *Glomus geosporum*, (B) Few arbuscules produced by *Glomus mosseae*, (C) *Glomus mosseae* produces few intraradical vesicles (ve), (D) Arbuscular infection by *Glomus versiforme*, (E) and (F) Abundant vesicle production by *Glomus versiforme*.

3.4.3.7 Photomicrographs of 48 day old wheat root showing infection morphology. (A) Abundant arbuscule (ar) production by *Glomus geosporum* and (B) Infection morphology of *Glomus mosseae* is characterized by small arbuscules and intraradical hyphae (h).
1 INTRODUCTION

Vesicular-arbuscular mycorrhizae (VAM) are a group of fungi that form obligate, mutualistic symbioses in roots of most terrestrial plants (Trappe 1987). This group of fungi contributes substantially to the establishment, productivity, and longevity of natural or man-made ecosystems (Harley and Smith 1983). A single fungus is capable of infecting a wide range of host species, indicating absence of host-fungus specificity (Mosse et al. 1981; Anderson 1988). However, because the growth response of different hosts to individual VAM differs, some degree of host specificity may exist (Bethlenfalvay et al. 1982; Koomen et al. 1987). Geographically, VAM are ubiquitous being found in tropical, temperate, arctic regions and in soils and crops of different agroecosystems (Hayman 1982a; Trappe 1987; Abbott and Robson 1991a; Allen 1991).

The mechanisms by which VAM interact with host plant to affect its growth and development in natural and agroecosystems are not well understood. Experimental evidence to demonstrate direct benefits of VAM to plants in natural ecosystems is scarce (Schenck and Sequeira 1987; Fitter 1989), and the importance of mycorrhizae is often ascribed simply to the abundance of the association (Allen 1991). However, the levels of VAM infection among plant species and between sites in different natural habitats vary considerably. Fitter (1989) hypothesized that host plants differ in the amount and time of P demand during their growth cycle, and that the observed variation in VAM infection levels is a reflection of the benefits obtained by host plants at times when their demand for P is much greater than the supplying capacity of their root systems. The importance of VAM in the natural ecosystem is evident because: (i) many plant species can not grow adequately without VAM fungi, especially in P-deficient soils (Hayman 1982a), (ii) many plant species may be eliminated from disturbed ecosystems where drastic reductions or removal of VAM propagules have occurred (Miller 1979; Reeves et al. 1979) (iii) VAM improve survival of plants transplanted to exotic environments (Allen 1991).
The importance of VAM in agroecosystems is best demonstrated by growing VAM inoculated plants in fumigated, P-deficient field soils. Under such controlled conditions, or in pot culture studies with sterile P-deficient soils, VAM infected plants out-yield controls several fold (Powell 1982; Hayman and Tavares 1985; Schubert and Hayman 1986). Enhanced crop growth by VAM inocula under natural field conditions also has been demonstrated by many workers (Saif and Khan 1977; Abbott and Robson 1981; Fitter 1985; McGonigle 1988).

How do vesicular-arbuscular mycorrhizae improve the growth of agricultural crops? Increasing the absorption of phosphorus is one key mechanism. Phosphate ions are relatively immobile (diffusion rates of $<10^{-8}$ cm$^2$ s$^{-1}$) in soils. The extramatrical VAM hyphae reach phosphate ions that can not diffuse to the roots, making them available to the host plant (Harley and Smith 1983). Extramatrical VAM hyphae may absorb as much as 80% of the plant P (Li et al. 1991a). In addition, mycorrhizal plants may also exhibit increased drought resistance (Nelson 1987; Bethlenfalvay et al. 1988; Ibrahim et al. 1990), uptake of some micronutrients such as Zn (Swaminathan and Verma 1979; Pacovsky 1986) and Cu (Gildon and Tinker 1983; Li et al. 1991b), tolerance to salt injury (Hirrel and Gerdemann 1980), nodulation of legumes by *Rhizobium* spp. (Young et al. 1988), and possibly greater resistance to diseases (Mosse et al. 1981; Linderman 1988). Vesicular-arbuscular mycorrhizae may also protect plants from toxicity of heavy metals such as Mn (Kothari et al. 1991). Thus there is a considerable interest in the possible utilization of mycorrhizal fungi as "biofertilizers" for agriculture (Menge et al. 1978a; Jeffries 1987; Howeler et al. 1987; Gianinazzi et al. 1989; Stribley 1989; Abbott and Robson 1991b). However, a better understanding of VAM ecology (Mosse et al. 1981) and their associations with the field crops is needed to effectively utilize these fungi in agricultural production systems (Fitter 1985; Howeler et al. 1987).
The conversion of land with native vegetation for agriculture probably disturbs the fine balance that existed between mycorrhizae and plants in the natural ecosystems (Schenck and Kinloch 1980; Schenck and Sequeira 1987). Furthermore, different agricultural practices may affect VAM community dynamics in agroecosystems (McGraw and Hendrix 1984; Miller et al. 1985; Schenck and Kinloch 1980; Howeler et al. 1987; Baltruschat and Dehne 1988; Abbott and Robson 1991a; Johnson et al. 1991a).

Together, these practices may result in the elimination of VAM strains from an ecosystem. Furthermore, it is possible that some of these VAM may be the most efficient ones in the ecosystems. Thus, in order to exploit the benefits of VAM, it may be necessary to (i) promote proliferation of efficient indigenous VAM strains present in field conditions, or (ii) isolate efficient VAM for production of inocula to be used in agricultural fields with low numbers (Clarke and Mosse 1981; Thompson 1987) or ineffective VAM (Medina et al. 1988).

Growth chamber experiments show that VAM strains differ in their ability to stimulate growth of agricultural crops (Mosse 1972; Sanders et al. 1977; Powell 1982; Jensen 1984a). Furthermore, the activity of VAM strains may vary depending upon crop species and the different sets of environmental conditions supporting growth of the host plant (Bethlenfalvay et al. 1982; Graham et al. 1982a; Hayman and Tavares 1985; Schubert and Hayman 1986; Koomen et al. 1987; Medina et al. 1988). This emphasizes the need for a better understanding of the different factors controlling the types, effectiveness and population dynamics of VAM propagules in soils of a particular agroecosystem. For example, a survey of different agroecosystems may identify local VAM endophytes which might prove useful as inoculants. Such studies might also identify and isolate VAM strains which stimulate growth of a broad range of agricultural crops grown under different conditions. For example, Howeler et al. (1987) used this strategy to isolate two VAM, *Glomus manihotis* Howeler, Sieverding and Schenck and
Entrophosphora colombiana Spain and Schenck, which were very effective on a range of crops grown under various nutrient regimes.

Little information is available regarding VAM in Saskatchewan. For example, Bakerspigel (1956) found spores of *Endogone fasciculatum* (now known as *Glomus fasciculatum*) in 35 soils of Saskatchewan and Manitoba. Pang and Paul (1980) isolated *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe from soils cropped with fababean (*Vicia faba* L.). Kucey (1980) and Kucey and Paul (1983) studied the occurrence of VAM spores in Saskatchewan soils and reported that VAM spores are present in significant numbers to be important in crop production. However, the composition of the VAM community in Saskatchewan soils and their impact on crops such as spring wheat (*Triticum aestivum* L.) and lentil (*Lens esculenta* L.) are not well documented. Studies from different agroecosystems show that these crops are infected by VAM under greenhouse and field conditions (Jensen and Jakobsen 1980; Singh and Singh 1986; Young et al. 1985; Vierheilig and Ocampo 1991), but the contribution of VAM to their growth and development under field conditions is not known. My study was undertaken to determine the occurrence of VAM species in agricultural field soils and their impact on wheat and lentil grown in Saskatchewan, Canada. My objectives were:

(i) To study the occurrence of VAM in wheat and lentil and to isolate different VAM from wheat field soils.

(ii) To assess the effect of crop rotations on VAM infection in field grown crops.

(iii) To develop a method to propagate monospecific culture of VAM strains isolated from Saskatchewan soils.

(iv) To study the effect of selected VAM inocula on growth of wheat and lentil.
2 LITERATURE REVIEW

2.1 A historical perspective of vesicular-arbuscular mycorrhizae (VAM)

In 1885, Frank coined the term "mycorrhizen" for the structures he observed in fungi-infected tree roots of a temperate forest. Prior to Frank, Kamienski observed the association of fungal hyphae with roots in 1881, and speculated on the function of this association by stating: "whatever was absorbed from the soil by root must pass through the fungal hyphae". Later, Frank distinguished two main types of mycorrhizae, ectotrophic (having a well-defined external sheath of fungal tissue enclosing the root i.e., ectomycorrhizae) and endotrophic (having inter-and intracellular penetration of the host roots i.e., endomycorrhizae) (Harley and Smith 1983). Thus the term "mycorrhizae" refers to the association of fungi with roots. Endomycorrhizae (or endotrophic mycorrhizae) are commonly known as vesicular-arbuscular mycorrhizae. Vesicular-arbuscular mycorrhizae (VAM) are the most common form of "mycorrhizae" observed in plants.

The nature of the fungal symbionts of endomycorrhizae remained obscure for a long period until the work of Mosse (1953). She isolated spores of mycorrhizal fungi from soil and used these spores to produce VAM infection in the roots of strawberry (Fragaria vesca L.) plants. This was the first demonstration that fungi in the family Endogonaceae formed mycorrhizal symbioses. Phillips and Hayman (1970) improved the techniques for staining mycorrhizal fungal structures in plant roots, and this greatly facilitated studies on the occurrence of endomycorrhizal fungi in different plant habitats. Over the last 25 years a large volume of work has improved our understanding about anatomy, physiology, and ecology of endomycorrhizae, and their potential as inoculants for crop production (Schenck 1982; Harley and Smith 1983; Powell and Bagyaraj 1984; Satir 1987; Allen 1991; Allen 1992).
2.2 VAM structures and their function in the symbiosis

The name "vesicular-arbuscular mycorrhizae" is derived from the characteristic structures, "arbuscules" and "vesicles". Arbuscules occur within the cortical cells of plant roots and "vesicles" occur within or between them. These two structures are used to distinguish VAM from other fungi in the rhizosphere. In addition to arbuscules and vesicles, the fungus also produces intraradical coenocytic hyphae (Fig. 2.1). Vesicles and arbuscules, along with intraradical coenocytic hyphae, constitute the vegetative phase of the fungus in the host roots and are important in the acquisition of carbon and nutrients for vegetative growth (Harley and Smith 1983; Bowen 1987). A portion of a VAM infected root may contain only hyphae, arbuscules, vesicles or mixture of these structures (Fig. 2.1). As a continuous link with the internal phase, the fungus produces an extensive network (Fig. 2.1) of hyphae into the soil. This network is believed to be responsible for increasing nutrient uptake by mycorrhizal plants.

The VAM fungus infects a host plant root either from a germinating spore, infected root pieces containing intraradical spores (e.g., Glomus intraradices Schenck and Smith) or intra- or extraradical hyphae present in soil. Before entering into the host cell the germinating spores produce an appressorium on the epidermal cells. Subsequently, the fungus invades the cortical cells through the epidermal layers and produces hyphae, arbuscules and vesicles. In general the production of arbuscules precedes production of vesicles during the growth cycle of the fungus.

Arbuscules are formed by dichotomous branching of intracellular hyphae. They are in contact with the host plasmalemma and take part in nutrient transfer between the host and the fungus. Arbuscules are more abundant in the cortical cells close to the vascular stele (Kinden and Brown 1975). A mature arbuscule functions for approximately four to fifteen days, after which it is digested (Cox and Tinker 1976; Bowen 1987).
Figure 2.1 Photomicrographs of infected root segments showing typical structures of vesicular-arbuscular mycorrhizae. (A) Vesicles (ve), arbuscules (ar), internal and extramatrical hyphae (h) and external chlamydospores (sp), and the root segments infected with (B) arbuscules, (C) vesicles, (D) internal hyphae and (E) extramatrical hyphal network (h).
Unfortunately, there is little information on the dynamics of arbuscule production by different VAM in a single host, or by a single VAM in different hosts.

It is known that various edaphic factors such as nutrient status and pH may influence the number of arbuscules produced by a VAM. For example, Braunberger et al. (1991) observed that *Glomus versiforme* (Kersten) Berch produced fewer arbuscules in maize (*Zea mays* L.) roots when P levels were increased. Other workers have reported similar results on the effects of P on the number of arbuscules formed in host roots (Mosse 1973a; Menge et al. 1978a; Amijee et al. 1989; Clapperton and Reid 1992). Thompson (1986) observed that *G. fasciculatum* (Thaxter sensu Gerdemann) Gerdemann and Trappe or *G. mosseae* (Nicol. & Gerd.) Gerdemann and Trappe produced different levels of arbuscules in wheat and maize at a given NO₃-N:NH₄-N ratio and different P levels. Schenck and Schroder (1974) observed increased arbuscule formation due to a decrease in temperature at which host plants were grown. Smith and Sukarno (1992) reported that two fungicides, Benomyl™ [methyl 1 (butylcarbonyl) benzimidazol-2 ylcarbamate] and Aliette™ (fosetyl ethyl hydrogen phosphonate aluminum) reduced the development and activity of arbuscules in onion (*Allium cepa* L.) roots.

Different combinations of VAM species in a soil may be associated with the different numbers of arbuscules produced in the host roots growing in that soil. For example, inoculation of western wheat grass (*Agropyron smithii* Rydb.) with soils collected at different slope positions (soils at different slope positions contained different VAM communities) resulted in different levels of arbuscule production in roots and P concentrations in shoots (Henkel et al. 1989). Trinick (1977) studied the infection morphology of *Lupinus* spp. grown in their natural habitat in the south-west coast of Australia and in a greenhouse, and observed that only a few species contained arbuscules in their roots. More studies are required on the types and amounts of arbuscules formed.
by different VAM species in different hosts and under different environmental conditions, particularly in field grown crops.

Most VAM fungi form vesicles in the host root, although the rate and pattern of vesicle development in mycorrhizal roots appear to vary among host species and with changing environmental conditions (Tommerup 1984; Morton 1985; Thompson 1986; Abbott et al. 1984). Vesicles appear to serve mostly as storage organs inside roots (Bonfante-Fasolo 1984) rather than as infective propagules, although the latter have been reported by Biermann and Linderman (1983). Trinick (1977) observed that 30% of the field grown plants of Lupinus spp. contained vesicles, whereas greenhouse grown plants rarely produced vesicles. In some Glomus species, vesicles appear to be transformed into thick-walled spores indistinguishable from extraradical spores (Morton 1985). Vesicle structures inside the host root may be characteristic of different VAM species, although some mycorrhizae do not produce intraradical vesicles (e.g., mycorrhizal fungi in the genera Gigaspora and Scutellospora). However, no studies have compared vesicle structures produced by different VAM in a single host.

2.3 Taxonomy of VAM

Morphology and germination characteristics of asexual spores are used to classify VA mycorrhizal fungi in the division Eumycota, class Zygomycetes, order Endogonales, family Endogonaceae (Trappe 1982). The family Endogonaceae was studied little before the discovery that its members formed VAM associations. The fungal partners of the endomycorrhizal symbiosis were placed in the genus Rhizophagus (of the family Endogonaceae) by Dangered as early as 1896 (Harley and Smith 1983), but none of the characteristics of Rhizophagus described the nature of the VAM isolates subsequently identified.
Thaxter (1922) divided the family Endogonaceae into four genera, *Endogone*, *Sphaerocreas*, *Sclerocystis* and *Glaziella*. Both ectomycorrhizal and endomycorrhizal fungi were included in the genus *Endogone* on the basis of formation of sporocarps by them (Nicolson 1967; Nicolson and Gerdemann 1968). However, later on it was known that the nature of the fungi in the genus *Endogone* that form ecto- and endomycorrhizae was different. Endomycorrhizal fungi produce asexual spores or azygospores [except the fungus in the species *Gigaspora decipiens* Hall and Abbott (Tommerup 1987)], whereas ectomycorrhizal fungi produce sexual spores or zygospores. *Endogone fasciculatum* and *E. microcarpum* were the first VAM species included in the family Endogonaceae (Thaxter 1922; Godfrey 1957). Subsequently, two new genera which formed VAM were added to family Endogonaceae: *Glomus* (Tulasne and Tulasne) and *Gigaspora* (Gerdemann and Trappe) (Harley and Smith 1983).

Fungi in the genus *Endogone* which form mycorrhizae consist of several diverse elements. Therefore, Gerdemann and Trappe (1974) published a monograph of the family Endogonaceae. In this monograph mycorrhizal fungi were categorized in five genera: *Acaulospora*, *Endogone*, *Glomus*, *Gigaspora* and *Sclerocystis* (Fig. 2.3.1). These genera are separated at the first level on the manner of spore formation, as inferred from the morphology of spores and spore-bearing structures. The monograph of Gerdemann and Trappe (1974) on Endogonaceae served as a "temporary" classification system for VA mycorrhizal fungi (Fig. 2.3.1) which provided an orderly framework for scientists to conduct research on VAM. It is now known that only *Glomus*, *Sclerocystis*, *Gigaspora* and *Acaulospora* are VA mycorrhizae. Since 1974, many new species of VA mycorrhizal fungi have been described based on the keys described in the "temporary classification system" (Fig. 2.3.1) and a new genus, *Entrophospora*, was proposed by Ames and Schneider (1979). Thus, at the present time, there are six genera in the family Endogonaceae which form VAM.
Two divisions of fungus

**Myxomycota** (Possess plasmodium)

**Eumycota** (True fungi)

Lower form of fungi without septation

**Zygomycotina**

**Mastigomycotina**

Higher form of fungi with septation

- Ascomycotina
- Basidiomycotina
- Deuteromycotina

Class **Zygomycetes**

Order **Endogonales**

Family **Endogonaceae**

Genera

- **Acaulospora** (2 species)
- **Glomus** (19 species)
- **Gigaspora** (5 species)
- **Sclerocystis** (4 species)
- **Endogone** (11 species)
- **Modicella**

Form vesicular-arbuscular mycorrhizae

Figure 2.3.1 Classification of vesicular-arbuscular mycorrhizae in the family Endogonaceae (Adapted from Gerdemann and Trappe 1974). The bold letters represent different taxa of fungi that form vesicular-arbuscular mycorrhizae.
Concepts on the taxonomy of VAM have been continuously changing so that we have a better evolutionary perspective about VAM, and also an understanding about their interactions with host plants (Berch 1986; Walker 1987a; Morton 1988; Pirozynski and Dalpe 1989; Morton 1990a; Morton 1990b). Recently, Morton and Benny (1990) revised the classification of VAM (Fig. 2.3.2) to make it more natural than the earlier system (Gerdemann and Trappe 1974). The revised classification considers characteristics which reflect the greatest physiological importance in an organism's life cycle and group taxa related by a common ancestor (Morton 1990a; Morton 1990b; Morton and Benny 1990). This classification system includes the 129 VAM species described thus far (Schenck and Perez 1988).

The question arises as to what is the practical purpose of VAM taxonomy? It is already described that a single fungus can infect a wide range of hosts indicating a lack of specificity between host and the fungus. Ecotypes (morphologically similar) of VAM species have been identified which are adapted to soils containing high concentrations of heavy metals (Gildon and Tinker 1983), salt (Allen and Cunningham 1983) and aluminum (Adelman and Morton 1986). These examples emphasize that the individuals of a species differ genetically from one another, or that environmental factors affect genetically similar members of the species differently. Thus ecotypes may differ in their symbiotic interaction with the hosts. Although an existing taxonomy may not be directly useful to understanding the types of interaction of VAM ecotypes with the host, it serves to identify VAM species from different geographic locations. Furthermore, the taxonomy provides a framework for scientists to compare results of experiments conducted to study interactions between VAM species (isolated from different geographic locations and identified as members of different taxonomic groupings) and different host plants.
Figure 2.3.2 Revised classification of vesicular-arbuscular mycorrhizal fungi in the class Zygomycetes (Adapted from Schenck and Perez 1988; Morton and Benny 1990).
2.4 Identification of VAM

Identification involves the practical use of information collected during classification of type strains of VAM fungi. For VA mycorrhizal fungi, this involves recognition of a spore or group of spores collected from a field or pot culture soil. In geographic areas where no previous identification has been made, this may involve determining if a VAM belongs to an established taxon or differs sufficiently to be described as a new species. For example, initial surveys of native VA mycorrhizal fungi in West Virginia resulted in the identification and classification of four new species (Morton 1986).

2.4.1 Criteria for VAM identification

The most important criterion in classification and identification of VAM is morphology. Physical characteristics are important because they can be visualized universally with a good light microscope. Identification is achieved by comparing isolates to preserved specimens, species descriptions, illustration and keys. The morphological characteristics of VAM used for identification are: (i) organization of spores; (ii) sporocarp morphology; (iii) morphology of intact spores; (iv) spore wall structures; (v) factors modifying spore morphology; (vi) morphology of the subtending hypha; (vii) occlusion of spores and (viii) morphology of the sporiferous saccule (Morton 1988).

Spores of VA mycorrhizal fungi were first thought to be arranged either in loosely-formed clusters, randomly dispersed in a loose or dense hyphal network or highly ordered around a hyphal plexus (Gerdemann and Trappe 1974). Emphasis on sporocarpic forms has been gradually replaced by an awareness that many VAM fungi (70%) form ectocarpic spores singly on hyphae (Morton 1988). Spores also can aggregate in animal remains (Rothwell and Victor 1984), insect husks (Rabatin and Rhodes 1982), or in roots (Morton and Walker 1984). In all but the last instance, spore
aggregation is more likely a response to the physical environment than to inherent genetic characteristics (Morton 1988).

Most VAM taxa forming spores in roots are in the genus *Glomus*. Almost any VAM fungus will produce an occasional spore in the root cortex, but some species, for example *Glomus aggregatum* (Schenck and Smith) koske or *G. intraradices* frequently form abundant intraradical aggregates of spores. Arrangement of spores in the sporocarp, the color, size, shape and surface texture of the sporocarp are important characteristics taken into consideration during identification of sporocarp-forming VAM species.

Different VAM taxa can often be separated visually under a low power dissecting microscope after spores are extracted from soil. Individual spores can then be separated by similarities or differences in color, size, and shape (Fig. 2.4.1). Accurate identification of each fungus requires careful examination of a number of spores, especially their wall structure, under a light microscope, often at high magnifications (Walker 1983).

The spore wall, more than any other morphological character, is used to identify a VAM. Wall structures provide important clues about vegetative growth, mode of reproduction, events in ontogeny of spores and survival mechanisms (Bartnicki-Garcia 1968). Spore wall structure always has been important in the classification and identification of VA mycorrhizal fungi. Wall types are identified strictly by the appearance of intact or broken spores under light microscopy. Walker (1983) first defined evanescent, unit, laminated and membranous wall types. Since then three new wall types have been described: the expanding wall (Berch and Koske 1986), the amorphous wall (Morton 1986) and the coriaceous wall (Walker 1986). The evanescent
Figure 2.4.1  Groups of spores belonging to: (A) *Glomus geosporum*, (B) *Glomus fasciculatum*, (C) *Glomus mosseae* and (D) *Glomus versiforme*. 
wall appears as a unit wall in young spores, and then breaks down or sloughs off as a spore matures. For example, an evanescent wall has been described in spores of *G. versiforme*, but it is difficult to evaluate because of its transitory nature (Morton 1988). The unit wall is defined as a rigid wall formed as a single layer which fractures easily when pressure is applied to a spore. Spores of *G. aggregatum* possess a unit wall. The laminated wall is also a rigid wall, but it is composed of fused multiple layers (referred to as "laminae") formed successively as the spore matures. It also breaks easily when pressure is applied to the spores. Spores of *Glomus geosporum* (Nicolson and Gerdemann) Walker, *G. mosseae*, *G. versiforme* and *Gi. decipiens* are examples of VAM with laminated walls.

The membranous wall is a thin, flexible wall formed as a single layer which often wrinkles or collapses in crushed spores. When a membranous wall is part of an inner wall group it does not fracture readily in spores broken with light pressure. This phenomenon has led to the term "endospores" being applied to an innermost membranous wall (Ferrer and Herrera 1981 as cited by Morton 1988). The membranous wall is found in spores of all genera of VA mycorrhizal fungi except *Sclerocystis*. The coriaceous wall is a thick, hyaline, flexible wall formed as a single layer. This wall type is termed "coriaceous" because of its leathery appearance, the outer surface appearing wrinkled in a hypertonic mountant. A few *Acaulospora* and *Scutellospora* species form this type of wall. The expanding wall is a hyaline unit wall which expands markedly when exposed to acidic mountants (e.g., polyvinyl-lactoglycerol, lactophenol). The expanding wall has been described in *Glomus pansihalos* Berch and Koske (Berch and Koske 1986).

Laminated, unit and membranous walls may possess surface topography or structures which give them an ornamented appearance. For example, the unit wall of *Acaulospora denticulata* Sieverding and Toro spores may be ornamented with echinulations consisting of polygonal tooth-shaped projections.
The number of spore walls varies among taxa in any genus of VA mycorrhizal fungi. In general, spores of *Glomus*, *Sclerocystis* and *Gigaspora* species usually have only 1-2 walls (80%, 100%, 100% of taxa in each genus, respectively). More than 3 walls are present in spores of taxa in *Acaulospora* (75%), *Scutellospora* (90%) and *Entrophospora* (67%) (Morton 1988). The number of spore walls is remarkably stable in most VA mycorrhizal fungi, being documented in *Glomus occultum* Walker and *Glomus diaphanum* Morton and Walker and inferred from observations of other taxa in different habitats (Morton 1985).

A wall group is defined as an aggregation of walls which remain in proximity to each other after a spore is crushed (Walker 1983). Such walls arrangements can be diagnostic, and are fairly consistent as long as spores are fresh, mounted in the same medium, and broken with an equivalent amount of pressure. Wall groups are best depicted in murographs (Fig. 2.4.2).

Melzer's reagent [(0.5 g iodine, 1.5 g potassium iodine, 20 mL chloral hydrate, and 20 mL water, (Morton 1988)] appears to offer the greatest potential as a stain to aid in the identification of VA mycorrhizal fungi. A positive reaction in Melzer's reagent results in a color change in specific fungal structures. A blue, or amyloid reaction results from reaction between iodine and starch. In Basidiomycete fungi, the amyloid reaction has been attributed to the binding of iodine with straight-chained α-1,4 glucosidic-linked polysaccharides. However, in spore walls of VA mycorrhizal fungi, a true amyloid reaction has not been observed. A red, or dextrinoid reaction occurs due to high concentrations of quaternary ammonium compounds (QACS), and is detected with the innermost membranous wall in spores of *G. fasciculatum* and *Acaulospora appendicula* Spain, Sieverding and Schenck. Spore walls of all other VAM fungi react with iodine forming a gradation of color between blue and red. These color gradations are related to
Figure 2.4.2  Murograph of wall type, number, group and position in a VA mycorrhizal fungus spore. (O) = ornamental surface, * = difficult to see. Lines connecting two walls depict a cementing layer. Walls numbered consecutively from the outer to inner surface. 1 = expanding wall, 2 = evanescent, 3 = laminated, 4-7 = unit, 8 = membranous, 9 = coriaceous, 10 = amorphous walls. Each wall group is bracketed in parentheses with consecutive letters from outer to inner surface (Adapted from Morton 1988).

the chemical composition of the spore walls (e.g., amylose, amylopectin, QAC).

Amylose, for example, reacts with iodine to form a purple to red-purple color when the degree of polymerization is between 30 and 40 glucose units. A faint red, red and blue reaction occurs with 12, 15 and 45 glucose units, respectively. In G. versiforme an orange or orange-red reaction is observed. The color reaction of inner walls with Melzer's reagent appears to be a very stable character (Morton 1988).
2.5 Occurrence and distribution of VAM

The presence of VAM in natural habitats and agroecosystems is determined by the occurrence of VAM infection in roots of different plant communities, or by detecting VAM spores and infective propagules in different soils. Although VAM are widely distributed, there is limited knowledge on the occurrence of individual VAM species in relation to soil, climate and vegetation (Hayman 1982a; Hayman 1982b; Abbott and Robson 1991a). Furthermore, the effects of such factors on the dynamics of VAM in different habitats may be useful in practical management of VAM.

Vesicular-arbuscular mycorrhizal fungi occur in almost all soils (Mosse et al. 1981; Hetrick 1984; Allen 1991). The few instances where these fungi may be absent include eroded soils (where surface soil containing the fungi is lost) or soils disturbed by mining (where either the removal of topsoil or disturbance eliminates the fungi). The VAM fungi are dispersed primarily by wind and soil transportation on either a small or large scale (Warner et al. 1987; Walker 1987b). Because of this ability to disperse and their extensive host range, it is not surprising that VAM fungi are widely distributed.

2.5.1 Distribution of VAM in the plant kingdom

VA mycorrhizae occur in most angiosperms as well as in some gymnosperms, pteridophytes and bryophytes. Most flowering plants have endomycorrhiza (almost entirely of the VA type) in contrast to only 3 per cent with ectomycorrhiza (Meyer 1973). Vesicular- arbuscular mycorrhizae are absent from only a few plant families, mainly those which form associations with ectomycorrhizae (Pinaceae and Betulaceae) or the two other specific types of endomycorrhiza (Ericales and Orchidaceae). However, biological boundaries are never rigid and some plant families (and even species) can form both ecto-
and endomycorrhizal associations, e.g., oak, hazel jupitor, sweetgum, poplar, tulip tree and some eucalyptus (Hayman 1982a).

In some plant families such as Cruciferae, Chenopodiaceae, Caryophyllaceae, Polygonaceae, Juncaceae and Cyperaceae the situation is confusing (Gerdemann 1968). Generally, plants belonging to these families do not form mycorrhizal symbioses (Harley and Smith 1983). However, there are occasional reports of VAM infections in some of these families (Kruckelmann 1975; Williams et al. 1974), but their appearance is not like typical VAM because arbuscules are lacking (Tester et al. 1987). Experimental evidence that plants in these families (e.g., Cruciferae, Chenopodiaceae etc.) are not truly VAM had been shown in studies where brassicas and chenopods (non-mycorrhizal hosts) were grown with or without a mycorrhizal "nurse" plant such as citrus (Citrus spp.), onion, maize, lettuce (Lactuca sativa L.), barley (Hordeum vulgare L.) or potato (Solanum tuberosum L.) (Hirrel et al. 1978; Ocampo et al. 1980). Small amounts of infection (hyphae, vesicles, but no arbuscules) developed in the non-host crops when a host nurse plant was present, but far less or even none developed in non-hosts grown alone.

2.5.2 Occurrence of VAM in major plant habitats

Geographically, VAM are ubiquitous, being present in tropical, temperate and arctic regions. For example, spores of the VAM species Acaulospora laevis Gerdemann and Trappe have been detected in Australia, Brazil, England, New Zealand, Pakistan, Scotland, South Africa and the U.S.A. (Hayman 1982a) Similarly, G. mosseae is known to occur globally in a broad range of dissimilar environments (Gerdemann and Trappe 1974; Koske 1987). This is difficult to explain as VAM spores are not readily airborne like conidia or rust uredospores, and therefore unlikely to be carried in air currents over vast distances. Limited dispersal can occur with water, soil animals and wind-blown soil dust (Walker 1987b; Warner et al. 1987), but broader dispersal is
usually accounted for by spread in association with the spread of host plants. Since the host range of VAM fungi compared to the other plant-infecting fungi is wide, dispersal with spread of host plants can be most common. Fossil plants from some 300 million years ago contain fungal structures interpreted by many as VAM (Pirozynski and Dalpe 1989). If this is so, then perhaps the VAM fungi had a very ancient origin and spread around the globe before separation of the continents and evolution of the phanerograms (Hayman 1982a).

Redhead (1968) found VAM in all 15 exotic and 44 out of 51 indigenous plant species that he examined in a lowland tropical rain forest in Nigeria. St. John (1980) reported an abundance of VAM species with dicotyledonous root systems in a Brazilian rain forest. Hayman (1982a) found VAM in every plant examined except the ectomycorrhizal trees in a deciduous woodland in England. Very wet areas such as rice paddy fields generally lack VAM, although Mejstrik (1972) found extensive endomycorrhiza in a marshy habitat. VA mycorrhizae are also abundant in many temperate grasslands (Wallace 1987). Crush (1973) found both VAM infections and VAM spores to be widespread in the native tussock hill grassland of New Zealand. Read et al. (1976) found all the most important plant species that they examined in the semi-natural hill grasslands in England were mycorrhizal, with most individual plants heavily infected, especially members of the Gramineae. Few VAM spores but extensive VAM infection of roots were found in acid hill grasslands in Northern England (Sparling et al. 1978) and the eastern United States and Canada (Molina et al. 1978).

The relationships between the level of mycorrhizal colonization in natural vegetation, and soil chemical and physical properties are markedly variable (Sparling et al. 1978; Newman et al. 1981). High levels of infection have been observed over a wide range of (i) soil pH values (Read et al. 1976); (ii) soil phosphate levels (Crush 1975; Hayman et al. 1976; Jeffries et al. 1988) and (iii) soil salinity levels (Gerdemann 1968).
2.5.3 Significance of VAM infection of plant communities in natural habitats

Most plants in natural habitats are VA mycorrhizal (Section 2.4.2). It is important to understand the contribution of VAM fungi to the function and productivity of plant communities in natural ecosystems, but direct evidence for the importance of VAM fungi is rare. Since VAM fungi are obligate symbionts, infection of plant roots exerts a metabolic load on the host plant. The general argument, therefore, is that if the host plant did not obtain any benefit from the fungus the symbiosis would have been eliminated from nature. Thus the importance of VAM is ascribed to their widespread occurrence in plant communities. There is, however, indirect evidence for the significance of VAM. For example, Miller (1979) studied the composition of mycorrhizal and non-mycorrhizal plant species in mine spoil sites where revegetation had occurred over many years, and in an adjacent undisturbed site where the natural vegetation still existed. They found that plant communities in adjacent sites consisted of naturally mycorrhizal and non-mycorrhizal plants. However, the number of non-mycorrhizal species was much higher in the mine spoil sites, indicating that non-mycorrhizal plants colonized the mine spoil sites during the revegetation period. It was argued that if VAM were not essential for mycorrhizal plants to colonize in mine spoil sites (from where VAM propagules are removed), then the plant community of these sites should have been similar to that of the adjacent undisturbed sites. Reeves et al. (1979) made similar observations on a community of sage plants in adjacent disturbed and undisturbed sites.

Data on VAM infections of plant roots growing in natural habitats suggest that infection varies with plant species, study sites and the growth stage of the plants from which samples are collected. Fitter (1989) hypothesized that since the level of infection is a reflection of the contribution of VAM to plants in natural habitats, therefore, the
benefits of the VAM symbiosis will vary depending upon the host species, site characteristics and the stage of crop growth. However, results of some field and growth chamber studies with agricultural crops indicate that the relative contribution of VAM to host growth and development is not always related to the magnitude of infection (Graham et al. 1982a; Abbott and Robson 1978; Vierheilig and Ocampo 1991). Thus Fitter's hypothesis may not be valid. Experimental evidence is required to arrive at definite conclusions about the significance of VAM in natural ecosystems. However, a lack of appropriate techniques has limited attempts to manipulate VAM populations in the field.

2.5.4 Effects of agriculture on the VAM of natural ecosystems

Few studies have assessed changes in the incidence of VAM species following conversion of native ecosystems to agroecosystems (Schenck and Kinloch 1980 and Schenck and Sequeira 1987; Johnson et al. 1991b). It is generally assumed that perennial ecosystems contain fewer VAM spores than cultivated fields subject to annual disturbance. This is because perennial ecosystems exert no evolutionary pressure to select VAM fungi that are able to produce long-lived resting spores necessary to survive periods without plant cover. In natural ecosystems the edophytes can survive from year to year inside living and dormant roots (Hayman 1982a). In contrast, agroecosystems exert selective pressures on VAM fungi because different agricultural practices are used from one year to the other. It is important, therefore, to understand the ecology of VAM in different agroecosystems.

2.5.5 Occurrence of VAM in agricultural soils and crops

2.5.5.1 VAM infection and spore numbers in cereals and legumes

The abundance of VAM fungi in cereals and legumes has been examined both in surveys of agricultural fields and in response to treatments imposed experimentally. Most
cereals and legumes grown in agricultural fields are infected with VAM (Sutton and Barron 1972; Sutton 1973; Hayman et al. 1975; Kruckelmann 1975; Strzemska 1975; Hayman et al. 1976; Iqbal and Qureshi 1976; Trinick 1977; Hayman and Stovold 1979; Jensen and Jakobsen 1980; Lambert et al. 1980; Jakobsen and Nielsen 1983; Young et al. 1985; Zajicek et al. 1986; Johnson et al. 1991a). In general, most VAM spores and infection of roots occur in the top 20 cm of soil. There is an exponential decline in both infection and spore numbers with depth (Sutton and Barron 1972; Redhead 1977). In most of these studies the occurrence of VAM was assessed at only one time during the growth cycle of the crop. However, variations in VAM infection during the entire growth cycles of different crops may be more important in understanding the contribution of VAM to crop growth, and also the influence of edaphic and climatic factors (Saif 1977) on VAM infection levels under field conditions (Hayman 1982a; Abbott and Robson 1991a).

There appear to be three phases in mycorrhizal development in annual cereals and legume under field conditions (Fig. 2.5.1). First is a lag phase where the percentage of infection increases slowly. In the second phase, lasting 30 to 35 days, extensive mycorrhizal development coincides with active shoot growth, resulting in a copious spread of external mycelium which leads to multiple infections. The third phase, from host fruiting to senescence, is characterized by a constant proportion of mycorrhizal to non-mycorrhizal roots (Sutton 1973). There are, however, many variations of this general picture (Jakobsen and Nielsen 1983; Buwalda et al. 1985). A lag phase may reflect the availability of only a small number of effective propagules and/or unfavorable conditions for mycorrhiza formation. The longer lag phase observed in winter cereals is presumably because of cold soil temperatures (Hetrick and Bloom 1988b; Jakobsen and Nielsen 1983). Some crop species and cultivars exhibit a very rapid rate of root growth
Figure 2.5.1 The development of mycorrhizae in (A) Bean (*Phaseolus vulgaris* L. cv. Topcrop) (Adapted from Sutton 1973), (B) Field pea (*Pisum sativum* L. cv. Bondi) (Adapted from Jakobsen and Nielsen 1983), and (C) Winter wheat (*Triticum aestivum* L. cv. Avalon) grown at G-1 site (Adapted from Dodd and Jeffries 1986).
relative to the capacity of VAM to infect the roots during the period from seedling emergence to flowering. This may result in less root length being infected during plant growth (Dodd and Jeffries 1986).

In a few instances mycorrhizal spore populations have been monitored during production of crops (Mason 1964; Khan 1972, 1975; Sutton and Barron 1972; Saif and Khan 1975). In annual crops, more VAM spores are found towards the middle or end of the growing season than at the beginning (Mason 1964; Hayman 1970; Smith 1980). This is attributed to increased spore production as root growth slows down or ceases.

A few studies have reported that the three phase pattern of VAM infection coincides with periods during which higher crop growth occurs and nutrients are taken up in larger quantities (Saif and Khan 1975). However, it may be difficult to make general conclusions about the positive contribution of VAM to crops at different stages of growth based on field data. This is because VAM infection of crop roots under field conditions reflects factors such as climate (rainfall distribution, soil temperature), root and shoot growth rate of crop species, and the ability of specific VAM species to infect roots. Many studies on VAM infection of field crops have not adequately described climate data or have omitted measurements of root and shoot growth (Abbott and Robson 1991a). In addition, few attempts have been made to distinguish the different species of VAM fungi prevalent in the field soils where crops are grown. Various agricultural practices such as fertilizer additions, lime application and acidification, pesticide applications and crop rotations also may cause variations in VAM infection of field grown crops. The influence of these factors on VAM infection of field grown crops should be studied thoroughly in order to be able to manage VAM during crop production.
2.5.5.2 Influence of soil properties on mycorrhizal status of field grown crops

Although many growth chamber studies have demonstrated that a clear relationship exists between levels of VAM infection and extractable soil phosphate (Hayman and Tavares 1985; Medina et al. 1988), such a relationship under field conditions is not frequently observed. For example, sites with large amounts of extractable phosphate may have high levels of infection and/or large number of spores, whereas sites with small amounts of extractable phosphate may have low levels of infection and/or low number of spores (Hayman 1978; Gianinazzi-Pearson et al. 1980; Young et al. 1985; Jeffries et al. 1988). In some studies a negative correlation has been found between the amount of extractable phosphate in soils and the abundance of VAM fungi as assessed by infection (Jensen and Jakobsen 1980; Bolgiano et al. 1983; Morita and Konishi 1989) or infectivity (Abbott and Robson 1982a). The reason why VAM infection of crops is not decreased at some field sites with high soil phosphate levels may be due to VAM species that are active under these conditions (i.e., high phosphate content). For example, VAM species tolerant to very high levels of soil phosphate have been detected under field conditions (Davis et al. 1984). In addition, some VAM species differ in the extent to which phosphate decreases mycorrhiza formation (Thompson et al. 1986). In fact, Hayman et al. (1976) attributed the lack of any correlation between VAM infection over a range of sites in southern Spain and soil fertility to the overriding importance of natural variability in propagule distribution.

2.5.6 Distribution of VAM species

The distribution of VAM species in different agricultural soils may vary depending upon soil type, the presence of different crops and agricultural practices. Thus there is a
need to study the occurrence of specific VAM species in different agroecosystems with varying soil conditions and diverse climatic parameters.

2.5.6.1 Techniques used to study the distribution of VAM species

Most field soils contain a mixture of VAM fungi associated with plant roots. Several simple steps are employed to determine the species composition of an agricultural field: (i) collection of a representative soil sample from the top 20 cm of a soil profile (most VAM spores occur in the top 20 cm of soil) (Sutton and Barron 1972; Redhead 1977); (ii) direct separation of spore mixtures from soil by wet sieving and decanting, followed by density gradient centrifugation (Smith and Skipper 1979; Daniels and Skipper 1982); (iii) separation of the spore mixture into groups of similar looking spores using a stereomicroscope; and (iv) confirmation of different spore groups by detailed observation of permanently mounted spores under a light microscope and using a standard taxonomic key (e.g., Schenck and Perez 1988).

Some investigators have noted that low spore numbers (INVAM Newsletter, 1990 Vol 1 Issue no. 1) and a hyperparasitization of indigenous VAM spores (Ross and Ruttencutter 1977; Daniels and Menge 1980) in field soils may hinder correct identification of VAM species. Therefore, it is recommended that VAM in field soils be propagated in the greenhouse prior to identification. In this approach, however, spores of some species which were present in the original inoculum may not be detected (they may be sensitive to unknown environmental factors related to the soil, pot environments, or greenhouse conditions) or spores which were not observed in the original inoculum may be detected (stimulated by the growth conditions) in the pot cultures (INVAM Newsletter, 1990 Vol 1. No. 1). Using these techniques VAM spores are readily detected in different soils, and presumably, reflect the varying community composition of the VAM species.
present. This community may reflect various factors such as the host crops, soil characteristics and the agricultural practices that characterize different agroecosystems.

2.5.6.2 Factors affecting distribution of VAM species

Agricultural soils are inhabited by a complex community of several (Abbott and Robson 1977a; Schenck and Smith 1981; Stahl and Christensen 1982; Berch et al. 1989) to more than a dozen VAM species (Schenck and Kinloch 1980; Hetrick and Bloom 1983; McGraw and Hendrix 1984; An et al. 1990; Johnson et al. 1991a; Ellis et al. 1992). However, there is no clear-cut relationship with respect to the occurrence of a particular VAM species and various soil parameters or host plants in all of these studies. Nevertheless, there are marked differences among VAM fungi in their response to different soil properties, which might influence their distribution and abundance. For example, some VAM fungi are restricted to either acid or alkaline soils, whereas others occur in both acid and alkaline soils (Table 2.5.1; Young et al. 1985; Porter et al. 1987; Robson and Abbott 1989).

In some agroecosystems, cultivation (tillage and fertilizer application) has led to fewer VAM species (Schenck and Kinloch 1980; Hetrick and Bloom 1983), whereas in others, agricultural use may lead to greater diversity (Abbott and Robson 1977a). Nitrogen fertilizer, for example, depressed the number of 'white reticulate' spore types more than "laminate" types in a sandy loam (Hayman 1978). The composition of the VAM community also is strongly influenced by the crops in the production system (Schenck and Kinloch 1980; McGraw and Hendrix 1984; An et al. 1990; Johnson et al. 1991a). For example, Schenck and Kinloch (1980) studied in detail the selective effects of different crops on VAM species. Three Gigaspora species were the most numerous VAM around soybean (Glycine max L.) roots, whereas the most prevalent with bahia
grass (Paspalum notatum Flugge) were two Glomus species, and with cotton (Gossypium herbaceum L.) and peanut (Arachis hypogaea L.), Acaulospora spp.

Crop rotations also are likely to influence the species composition of the VAM community. For example, G. aggregatum, Glomus leptotichum Schenck and Smith and Glomus occultum Walker spores were more abundant in soil with a history of maize than a history of soybean, whereas spores of Glomus microcarpum Tul and Tul exhibited the reciprocal pattern (Johnson et al. 1991a). An et al. (1990) and An (1991) found increased diversity of VAM species in a rotation of soybean and maize, milo (Sorghum vulgare L.), or tall fescue (Festuca arundinacea Schreb). Recently, Sanders and Fitter (1992) reported evidence of differential responses between host-fungus combinations of VAM from a grassland.

2.5.7 Influence of agricultural practices on VAM spore numbers in soils and VAM infection in field grown crops

The mycorrhizal status of agricultural soils, in terms of crop root colonization and spore numbers, is affected by various agricultural practices. These include tillage, fertilizer amendments, lime application, soil acidification, agricultural chemicals

Table 2.5.1 The effect of pH on the distribution of spores of VAM fungi in the Merredin area of Western Australia (Adapted from Porter et al. 1987)

<table>
<thead>
<tr>
<th>Soil pH</th>
<th>Glomus sp. (WUM 2)</th>
<th>Glomus sp. (WUM 3)</th>
<th>Glomus monosporum</th>
<th>Scutellospora spp.</th>
<th>Acaulospora laevis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5.0</td>
<td>0</td>
<td>0</td>
<td>60</td>
<td>0</td>
<td>80</td>
</tr>
<tr>
<td>5.0-6.0</td>
<td>0</td>
<td>0</td>
<td>70</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>6.1-7.0</td>
<td>25</td>
<td>25</td>
<td>75</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>&gt;7.0</td>
<td>100</td>
<td>57</td>
<td>28</td>
<td>0</td>
<td>14</td>
</tr>
</tbody>
</table>
and crop rotations. Tillage methods appear to have smaller effects on the number of VAM spores than on the distribution of spores with depth. For example, although ploughed soils contain more spores in the 8-15 cm layer and fewer spores in the top 8 cm, the number of VAM spores in the 0-15 cm depth of ploughed and direct drilled plots is similar (Smith 1978a). Kruckelmann (1975) also found more spores in the top 7 cm of soil of direct drilled plots than in soil that had been ploughed.

Soil disturbance can decrease mycorrhizal infection (Evans and Miller 1988; Fairchild and Miller 1988; Jasper et al. 1989). Soil disturbance is caused by the tillage operation, the intensity of the operation determining the extent of disturbance. Phosphate uptake by plants after ploughing the soil (compared to plants grown on no-till soil) is decreased, and may be associated with the effects of soil disturbance on the formation of mycorrhizas (O'Halloran et al. 1986). Further evidence to support this hypothesis is that soil disturbance decreased phosphate uptake by plants that form mycorrhizas (wheat and maize), but not of plants [spinach (Spinacea oleracea L.) and rape (Brassica juncea var. toria)] that do not form mycorrhizas (Evans and Miller 1988). Furthermore, Jasper et al. (1989) showed that soil disturbance decreases the infectivity of VAM fungi by disrupting the hyphal network in soil.

The length of infected root for plants grown without tillage was almost twice that in plants grown with mouldboard ploughing, but there was only a small effect in the top 7.5 cm (Anderson et al. 1987). Increasing the intensity of the tillage operation decreased mycorrhiza formation in dry beans (Phaseolus vulgaris L.). This was apparently associated with increased soil compaction and decreased root growth (Mulligan et al. 1985). The practice of minimum tillage in combination with herbicide use at recommended rates to control weeds appeared to have a favorable effect on the formation of mycorrhizas (Smith 1978b).
Changes in soil fertility due to amendments with mineral fertilizers can markedly affect the activity of the soil mycorrhizae by influencing the level of root infection and spore production. Adding phosphate fertilizers either had no effect (Porter et al. 1978; Anderson et al. 1987) or decreased (Hayman et al. 1975; Jensen and Jakobsen 1980; Jensen 1983; Kucey and Paul 1983; Jakobsen 1986) VAM infection in a range of agricultural crops (Table 2.5.2). Few studies have assessed the effects of nutrients, other than phosphorus, on VAM infection and spore numbers under field conditions. Nitrogen applications to wheat decreased VAM spore numbers and mycorrhizal infection (Hayman 1970, 1975), but its effect on VAM infection of barley varied greatly at different field sites (Jensen and Jakobsen 1980).

In spite of the marked effects of soil pH on mycorrhiza formation by certain VAM fungi under greenhouse conditions (Skipper and Smith 1979; Robson and Abbott 1989), relatively little is known about the effects of either lime applications or soil acidification on the colonization of roots by mycorrhizal fungi in the field. Changing soil pH from 4.5 to 7.5 by adding lime did not affect the length of mycorrhizal root, but instead affected the fungal species responsible for infection (Wang et al. 1984). Plant roots were infected by VAM with fine hyphae in the unamended acid soil (pH 4.5), whereas roots were colonized by VAM fungi with coarse hyphae in the amended soil (pH 7.5). Thus, it seems that changes in soil pH affect the proportion of root colonization by a particular VAM, but are unlikely to change the total extent of VAM colonization (Abbott and Robson 1991a).

As for many other management practices, there have been few studies on the effects of agricultural chemicals on mycorrhizal infection in controlled field experiments. Results of greenhouse experiments suggest that use of agricultural chemicals (insecticides, fungicides and herbicides) at recommended rates will have minor effects on VAM (Smith 1978a). Nevertheless, in some situations it seems that herbicides applied at recommended
The effect of applying phosphate on colonization of barley roots by VAM fungi at five field sites in the Netherlands

(Adapted from Jensen and Jacobsen 1980)

<table>
<thead>
<tr>
<th>Site</th>
<th>Concentration of resin extractable P (μg g⁻¹ soil)</th>
<th>Amount of P applied (kg ha⁻¹)</th>
<th>Grain yield (kg ha⁻¹)</th>
<th>Root length colonized by VAM (%)</th>
<th>Root cortex colonized by VAM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.8</td>
<td>0</td>
<td>1770</td>
<td>74</td>
<td>43</td>
</tr>
<tr>
<td>2</td>
<td>12.4</td>
<td>0</td>
<td>5170</td>
<td>77</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>24.7</td>
<td>0</td>
<td>5100</td>
<td>56</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>19.3</td>
<td>0</td>
<td>4840</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>2.4</td>
<td>0</td>
<td>4120</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>
rates may decrease mycorrhizal colonization of roots (Dodd and Jeffries 1989). Some nematicides may increase VAM infection (Bird et al. 1974), but insecticides generally decrease it (Kruckelmann 1975). Smith and Sukarno (1992) found that the fungicide Benomyl acted directly on the VAM fungus, as the application of the fungicide reduced the growth of mycorrhizal onion (compared to control mycorrhizal plants to which Benomyl had been applied). It had little effect on non-mycorrhizal onion. Another fungicide, Aliette, acted both on the plant and the fungus as reflected by reduced (i) root growth of non-mycorrhizal onion (compared to growth of non-mycorrhizal onion without the fungicide) and (ii) fractional infection, external hyphae and arbuscule development in mycorrhizal onion to which aliette had been applied. However, the susceptibility of different VAM species to fungicides may differ (Spokes et al. 1981; Smith and Sukarno 1992).

Crop rotations followed in different agroecosystems have a distinct influence on VAM infection of host crops and spore numbers in agricultural soils. These rotations include continuous monoculture of host crops, a non-host crop in between host crops, the sequence of different host crops over the years, and a fallow break in monoculture for conservation of moisture and nutrients. Most studies on the effects of rotation on VAM activity have dealt with the absence of host plants for a period of time. More spores are found in continuously cropped soils than in soils maintained as clean fallows (Black and Tinker 1979; Kucey and Paul 1983; Thompson 1987). Similarly, mycorrhizal infection was greater in continuous crops than in crops grown after a fallow (Black and Tinker 1979; Thompson 1987). However, the effects of fallowing on mycorrhizal infection appear to be transient. There was no difference in the percentage of mycorrhizal infection of barley grown after two successive barley crops and barley grown after barley preceded by a fallow (Black and Tinker 1979). Nevertheless, barley grown after 2 fallow years had VAM infection levels only one-quarter of that in barley grown after two successive
barley crops. Few studies have stressed how quickly VAM infectivity declines in fallowed soils, or have made little attempt to separate effects of disturbance (associated with tillage) from those associated with the absence of host plants.

Host plants influence the occurrence and activity of VAM in soil. After 16 years of monoculture there were nine times as many VAM spores in soil planted with oats than in soil planted with the non-mycorrhizal sugar beet (*Beta vulgaris* L.) and black mustard (*Brassica juncea* L.) (Kruckelmann 1975). Fewer VAM spores were found in soil cropped with non-mycorrhizal plants such as crucifers, than mycorrhizal crops (Black and Tinker 1979). Such differences are unlikely to be attributed to the effects of residues of non-mycorrhizal plants (Ocampo and Hayman 1981). However, mycorrhiza formation, although initially less in crops grown after non-mycorrhizal plants, later increased to levels similar to those in crops that succeeded mycorrhizal plants (Ocampo 1980; Jakobsen 1986).

Differences in crop susceptibility to VAM presumably explains the observed changes in VAM populations with different rotations. For example, cassava (*Manihot esculenta* Crantz) is not heavily infected with VAM if there are low levels of VAM propagules in soil and therefore, when it is grown in monoculture, VAM infection of cassava roots remain low. However, cassava roots were heavily infected by VAM when grown in rotation with ground nuts (highly susceptible to VAM) which leave behind a high propagule density (Sieverding and Leihner 1984).

The interaction of soil microflora with VAM may also result in differences in infection level of VAM in field grown crops (Linderman 1991). Although field studies to show such effects are scarce, Wilson et al. (1988) reported that soils contain microorganisms that inhibit formation of mycorrhizae. The mechanisms involved in this suppression are not known, but early colonization dynamics, beginning with spore or
other propagule germination, are most likely to be involved. For example, it has been shown that spores of *G. versiforme* are colonized by bacteria which either stimulate or inhibit spore germination (Mayo et al. 1986). Similarly, certain bacteria have been shown to stimulate or inhibit hyphal growth of VA mycorrhizal fungi (Azcon-Aguilar and Barea 1985; Azcon-Aguilar et al. 1986; Azcon 1987; Mugnier and Mosse 1987; Hetrick et al. 1988a).

Some reports indicate that stimulatory bacteria produce volatile compounds that affect spore germination (Mugnier and Mosse 1987) and germ tube orientation relative to plant roots. Therefore, ecological or cultural factors that influence the microbial balance of a soil may favour mycorrhiza stimulators or inhibitors and may change the level of VAM infection under field conditions.

### 2.6 Production of inocula of VAM isolates

Production of inocula for large scale field use is often considered a handicap in practical application of VAM fungi for agriculture. Although VAM are obligate symbionts and can not be cultured on laboratory media (Mosse 1973a), methods of producing VAM inocula have gradually improved. These include mixed inocula of naturally infected soil or roots to inocula of known strains of endophytes raised from surface sterilized spores in association with a host plant growing in sterilized sand (Menge 1984).

Methods to produce VAM inocula are as varied as the researchers themselves. VAM inoculum production often resembles an art rather than true science because it requires manipulation of VAM fungi, rooting media, hosts, fertilizers, and environmental conditions (Menge 1984). However, these studies have identified many important variables that control inoculum production of different VAM species. Under greenhouse conditions, sporulation of VAM fungi can be influenced by host crops (Bagyaraj and Manjunath 1980a; Struble and Skipper 1988), nutrient levels (Menge et al. 1978a; Douds
and Schenck 1990), growth medium (Coltman et al. 1988; Sylvia and Jarstfer 1992) pot size (Ferguson 1981), temperature (Furlan and Fortin 1973; Schenck and Schroder 1974), light intensity (Ferguson and Menge 1982; Johnson et al. 1982) and initial inoculum doses (Ferguson 1981).

The VAM propagules which produce infection in a host plant may be spores, hyphae or hyphae from infected root pieces. Cultures of a VAM isolate usually contain a mixture of these propagules. Most studies on VAM inoculum production assess the effects of various factors on the number of spores produced in the rhizosphere of host crops. However, spores alone do not reflect the inoculum potential of a VAM culture. The inoculum potential of VAM cultures may be affected differently than those factors which influence spore production. The growth response of host crops to a VAM inoculant may be different depending upon the form of infective propagules assessed. For example, Hall (1976) found that G. mosseae infected root segments were more efficient than spores in stimulating growth of coprosma (Coprosma robusta.L.). Similar results were obtained by other workers (Abbott and Robson 1981). However, chlamydomspores are an important component of VAM inocula for increasing crop growth (Manjunath and Bagyaraj 1981). Thus I will review the effects of different factors on spore production in VAM culture.

Isolation of local endophytes and their establishment in pot culture is the starting point for application of VAM in local agricultural systems (Hayman 1982a). The indigenous VAM are better adapted to local conditions (Schenck at al. 1975) and are likely to be more effective than VAM collected elsewhere. An important consideration in the practical application of VAM in crop production is the identification of VAM isolates with more potential to stimulate growth at low spore densities. For example, Ferguson (1981) evaluated spore production and growth stimulation ability of three VAM species, Glomus constrictus Trappe, Glomus deserticola Trappe, Bloss and Menge and G. fasciculatum, at
different spore densities. Although *G. constrictus* was highly infective and stimulated growth of Brazilian sour orange (*Citrus auranticum* L.) to a greater extent at a very low spore density, its commercial potential was lower than *G. deserticola* because of its limited sporulation ability. Thus in addition to evaluating the efficacy of VAM isolates to promote crop growth, attention should be given to factors affecting spore production.

### 2.6.1 Factors affecting production of VAM inocula

Since most plants are mycorrhizal, a wide variety of plants are available for use to produce inoculum. Some host crops which have been used include mat-grass (*Nardus stricta* L.), coprosoma, citrus, sorghum (*Sorghum vulgare* L.), pencil-flower (*Stylosanthes* sp.), *Coleus* spp., onion, bell pepper (*Capsicum annuum* L.) strawberry, barley, maize, alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.), peanut and *Asparagus* spp. (Menge 1984). Bagyaraj and Manjunath (1980a) assessed the suitability of eight grasses for mass production of VAM inoculum, and found guinea grass (*Panicum maximum* Jacq.) to be superior to other species tested, including sorghum. Ferguson (1981) also assessed a variety of host plants and found both peanut and alfalfa superior to other crops for VAM spore production. Kormanik (1980) and Hetrick and Bloom (1986) reported sorghum to be the best host for production and maintenance of VAM inoculum. On the other hand Struble and Skipper (1988) found maize to be a better host than sorghum for production of *Glomus macrocarpum* Tul. and Tul., *Glomus claroideum* Schenck and Smith and *Glomus etunicatum* Becker & Gerd. spores. However, Ferguson (1981) found that the general health and vigor of the host plant may be more important in VAM inoculum production than the plant species.

The reasons why some hosts support greater spore production than others are not well understood. A higher level of root production (Ferguson 1981) and VAM infection of roots (Bagyaraj and Manjunath 1980a; Hetrick and Bloom 1986) have not been
associated with higher levels of spore production. Kormanik et al. (1980) suggested that increased VAM sporulation observed with sorghum compared to maize was due to the finer root system of sorghum. The quantity and type of root exudates produced by different hosts also may be associated with VAM sporulation. There may be differences in the interactions between host crops and different VAM species which result in different levels of sporulation. For example, Hetrich and Bloom (1986) found that spore production by three VAM on five host crops [asparagus (Asparagus officinalis L.), marigold (Tagetes erecta L.), red clover (Trifolium pratense L.), sudan grass (Sorghum vulgare cv. sudanense) (Piper) Hitchc., tomato (Lycopersicon esculentum L.) Mill.] was: G. macrocarpum > G. mosseae > G. fasciculatum, except that G. fasciculatum produced more spores than G. mosseae on sorghum. Furthermore, they observed that spore production of G. fasciculatum was significantly influenced by the host plant, whereas that of G. macrocarpum and G. mosseae was not.

Plant nutrition is probably an important factor influencing VAM sporulation. Phosphorus is shown to be important in controlling the level of VAM spore production in the rhizosphere of different hosts. Phosphorus additions reduce VAM colonization of roots and spore production in most soils (Jasper et al. 1979). Ratnayake et al. (1978) hypothesized that high soil phosphorus levels reduce mycorrhizal infection (and possibly spore production) because of a membrane-mediated decrease in root exudation. Graham et al. (1982b) also showed that a decrease in membrane-mediated root exudation due to higher P application was responsible for inhibition of mycorrhizal formation.

In addition to P, nitrogen stress may also influence the formation of VAM spores. For example, Douds and Schenck (1990) compared both the colonization and sporulation by Gi. margarita on bahia grass after addition of Hoagland solution with high levels of NH4NO3. Increasing the N in the Hoagland solution reduced both colonization and sporulation. However, it may not be possible to recommend suitable P and N levels for
host plants to achieve maximum VAM spore production. This is because plants vary in their ability to absorb P and mycorrhizal fungi vary in their response to P. Therefore, each plant-soil-VAM symbiont system must be evaluated separately. For example, in one sandy soil, 50 ppm added P allowed maximum spore production by *G. fasciculatum* on Brazilian sour orange, whereas with a different citrus, Troyer citrange (*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osbeck) no spores were produced (Ferguson 1981).

Temperature has a major effect on both host plant and VAM fungus growth (Furlan and Fortin 1973; Graham et al. 1982b; Schenck and Smith 1982). In most cases VAM colonization and spore production increase with increasing temperature until growth of the host plant is severely inhibited. For example, Ferguson (1981) found that spore production by *G. fasciculatum* on sudangrass increased as temperature increased up to 30°C which is the optimal temperature for growth of sudangrass. It is hypothesized that high temperatures increase both root exudation and VAM fungus growth which lead to increased VAM colonization (Smith and Bowen 1979; Graham et al. 1982b; Borges and Chaney 1989). Temperatures below 15°C are usually inhibitory to VAM colonization.

High light intensities and long daylengths improve mycorrhizal colonization or spore production in maize, alfalfa, sudangrass, citrus, onion, and many other plants (Daft and El-Giahmi 1978; Ferguson and Menge 1982). The increased VAM colonization apparently results from increased photosynthesis which leads to increased carbohydrate concentration in roots and (or) increased exudation of these compounds. Therefore, to maximize VAM inoculum production it would seem reasonable to attempt to maintain high light intensity in the growth chambers used. Most greenhouse or growth chambers provide only a fraction of full sunlight. Photoperiod extension seems to increase VAM colonization to a greater degree than does increasing light intensity. For example, reducing light intensity by 30% did not reduce mycorrhizal infection by *G. macrocarpum* on sudangrass (Johnson et al 1982), whereas by increasing photosynthetically active
radiation to sudangrass by 3-fold (with high intensity metal halide or mercury vapor lamps and extended photoperiods) spore production by *G. fasciculatum* was increased 5-fold (Ferguson and Menge 1982). Furthermore, commercial VAM inoculum can be produced in 2 months with this method instead of the normal 3 or 4 months. Sylvia and Jarstfer (1992) reported a aeroponic culture method for commercial inoculum production.

The size of growth pots or substrate volume also influences VAM spore production possibly by helping the host plant to produce more root mass which in turn increases spore production. Ferguson (1981) found that VAM spore production, both on a per gram of soil and per gram of root basis, increased with pot size. Plants in 15,000 cm³ pots produced 90 times as many spores per pot as in 750 cm³ pots. However, in this study, spore production on a per gram basis increased five times when pot volume was increased 20 times.

Vesicular-arbuscular mycorrhizal spore production can be enhanced by increasing light intensity, photoperiods, P and N stress. These factors may enhance spore production perhaps by governing the quality and quantity of root exudates in the rhizospheres of host plants. Since VAM species may differ in their sporulation behavior, these parameters should be assessed for a given VAM species to maximize production of spores.

### 2.6.2 Factors affecting the viability of VAM inocula

VAM inoculum production and maintenance is a laborious and time consuming process. Therefore, one important goal is to maintain the viability of the VAM inoculum for long periods following production. However, viability of VAM spores or other forms of propagules (e.g., infected roots, hyphae) may decrease with time as determined by their germination on water agar (Sylvia and Schenck 1983; Safir et al. 1990) and (or) by the level of infection in a host crop (Daft et al. 1987). The reasons why the viability of
spores decreases are not understood. Hyperparasitization of VAM spores stored for long periods may reduce the viability of spores as determined by their germination on water agar (Daniels and Menge 1980; Sylvia and Schenck 1983). Storage conditions are known to affect the viability of VAM spores (Daniels and Menge 1980; Sylvia and Schenck 1983; Daft et al. 1987; Gemma and Koske 1988; Safir et al. 1990). In general, viability of VAM inoculum is preserved for a long period (up to 4 years) if it is stored at 5°C (Ferguson and Woodhead 1982). One reason why spores may retain their viability over long periods under low temperature may be reduced hyperparasitization of VAM spores (Ross and Ruttencutter 1977). However, the optimum temperature for storage of VAM inocula from different climatic regions may be different (Mugnier and Mosse 1987).

On the other hand the storage of some VAM species spores following inoculum production is essential for retaining the infectivity of these spores. This is because some VAM isolates exhibit an innate dormancy [i.e., the inability to germinate and produce infection for a definite period of time after their production (Tommerup 1983)]. The VAM spores with an innate dormancy are viable, but not infective until the innate dormancy is broken. The low temperature at which VAM inocula are stored may break the innate dormancy of the spores and increase infectivity (Safir et al. 1990; Gemma and Koske 1988). This in another mechanism by which storage temperature may affect spore infectivity. Thus it is important to understand the effect of storage conditions on the viability and infectivity of VAM spores.

2.7 Plant growth response to VAM inocula

In general, VAM inoculants increase growth and yield of crops. This has been demonstrated repeatedly in pot experiments using sterile soil with low P levels and appropriate VAM inoculants. The magnitude of the crop growth response to VAM inoculants is determined by comparison to an uninoculated control. Monospecific VAM
inocula normally include a variety of associated microorganisms. The presence of these other soil microorganisms may influence the physiology of the mycorrhizal fungi (Linderman 1988; Linderman 1991; Will and Sylvia 1990). Some studies have used surface-sterilized spores to inoculate plants (uninoculated plants constituted the controls) (Meyer and Linderman 1986; Modjo and Hendrix 1986), but this technique of eliminating associated microorganism from VAM spores is impractical for large experiments. Instead, soil containing infected roots, hyphae and spores (or just spores) along with contaminating microorganisms are used to inoculate treated plants. The uninoculated control plants receive sievings obtained by passing suspension of spores or VAM inoculum mixture through a millipore filter that retains fungal inoculum but lets other contaminating microorganisms pass thorough. This precaution is taken to ensure that contaminating microorganisms are not responsible for enhanced or retarded plant growth in experiments designed to assess the effect of VAM inoculation (Abbott and Robson 1984a, b; Hetrick et al. 1988a; Koide and Li 1989).

2.7.1 Effect of VAM inoculation on growth and yield of crops in sterilized soil

Numerous workers (Hall 1978a; Carling and Brown 1980; Abbott et al. 1984b; Jensen 1984a, b; Hayman and Tavares 1985; Koomen et al. 1987; Waterer and Coltman 1987) have reported a positive growth response (of varying magnitude) of many crops to VAM inoculation in sterilized soil. For example, Hayman and Tavares (1985) found a 13-fold and a 6-fold increase in dry matter production of strawberry plants due to inoculation with *G. fasciculatum* and *G. mosseae*, respectively. Abbott et al. (1984a) found a 21% increase in the fresh shoot weight of 6 week old subterranean clover (*Trifolium subterraneum* L.) inoculated with a 100 g mixture of *G. fasciculatum*-infected white clover roots and soil from a pot culture. Daniels and Menge (1981) evaluated the commercial potential of *Glomus epigaeus* Daniels and Menge on a number of crops.
They found a 96% and a 208% increase in the dry weight of 4 month-old tomato and 5 month old asparagus plants, respectively. Soybean and sudangrass (Sorghum vulgare Pers.) did not respond to inoculation with G. epigaeus as well as tomato and asparagus. Jensen (1984a) observed a 100% yield increase in barley due to inoculation with G. constrictus, G. fasciculatum isolate no. 185 or G. fasciculatum isolate no. 0-1. Haas and Krikun (1985) found a 326 and 440% increase in foliage dry weight of 7 week-old bell pepper (Capsicum annuum L.) inoculated with 9 and 15 mL, respectively, of a G. macrocarpum soil culture.

2.7.2 Effect of VAM inoculation on growth and yield of crops in natural soil containing indigenous VAM

A number of studies have reported a positive growth response of many different crops to VAM inoculation of field soils (containing either ineffective indigenous VAM or low density of propagules) (Mosse 1977; Saif and Khan 1977; Black and Tinker 1979; Bagyaraj and Manjunath 1980b; Barea et al. 1980; Fitter 1985; Medina et al. 1988). For example, Medina et al. (1988) observed a 100% increase in the shoot dry weight of 70-day-old greenhouse grown bean (Macroptilium atropurpureum Urb. cv. Siratro) by inoculating plants with 240 propagules of Glomus etunicatum Becker and Gerdemann. The plants were grown in a greenhouse. Similarly, inoculation of plants with G. intraradices and G. deserticola (at equivalent propagule density) increased shoot dry weight ca. 60% and 19%, whereas G. versiforme and Gi. margarita had no significant effect on plant growth. Clarke and Mosse (1981) studied the effects of inoculation of barley with three VAM [(e.g., G. mosseae, Glomus caledonius (Nicol. and Gerd.) Trappe and Gerdemann G. fasciculatum)] in fields with low VAM propagules. Each of these VAM inoculants increased seed dry weight by 100%. Saif and Khan (1977) observed a 4-fold increase in the yield of barley grown in field soils low in available P (11.9-15.3 ppm) due to inoculation with VAM. The barley seedlings were pre-inoculated
with the VAM inoculum containing two species. Finally, McGonigle (1988) analyzed 78 published VAM field trials, and reported that there was on average a 37% yield increase of field grown crops and pasture herbs due to inoculation with VAM.

2.7.3 Effect of VAM inoculation on plant root growth

There is no clear relationship between the ability of a VAM inoculant to enhance shoot growth and yield of host crops and its effect on root growth. For example, *Gigaspora pellucida* Nicolson and Schenck increased shoot but reduced the root growth of soybean grown at 24°C, whereas *G. claroideum* increased both shoot and root growth under similar conditions (Schenck and Smith 1982). The reasons why some VAM species increased root:shoot ratios (Medina et al. 1988; Hung et al. 1990), whereas others decreased root:shoot ratios (Abbott et al. 1984a; Hall et al. 1984) are not known.

Bethlenfalvay et al. (1982) found that the root:shoot ratio of sorghum (*Sorghum bicolor* L.) and soybean inoculated with *G. fasciculatum* or *G. mosseae* decreased in all the soils tested except one. Price et al. (1989) found that inoculation of cotton with 250 spores of *Gigaspora margarita* Backer and Hall or *G. etunicatum* reduced the specific root length (cm root g⁻¹ fresh root weight) of mycorrhizal plants compared to that of the uninoculated plants. Hung et al. (1990) also reported a decrease in root length of mycorrhizal plants due to inoculation with eight separate VAM isolates. Kothari et al. (1990b) observed a 16, 31, 41, and 43% decrease in root dry weight, root length, root hair density and root hair length, respectively, of *G. mosseae* inoculated maize. In contrast to these studies Douds and Chaney (1986) observed an increase in the root dry weight of 16 week old green ash (*Fraxinus pennsylvanica* March) inoculated with 300 spores of *G. macrocarpum*. These results indicate that the effects of VAM inoculation on root growth and morphology may vary depending upon host-fungus-environment combinations (Bethlenfalvay et al. 1982).
2.7.4 Efficacy of VAM isolates to promote crop growth

Krishna and Dart (1984) studied the effects of six VAM fungi on the growth of pearl millet \textit{(Pennisetum americanum} Leeke) in sterile soil in a greenhouse. They found that the six species differed in their ability to increase shoot dry weight as follows: \textit{Gi. calospora} \textgreater \textit{G. fasciculatum} = \textit{Gi. margarita} > \textit{G. mosseae} > \textit{A. laevis} >>> \textit{G. fasciculatum} E3. The increase in shoot dry weight (over the uninoculated control) of the plants inoculated with these species ranged from 14 to 46%. On the other hand inoculation of plants with the \textit{G.fasciculatum} E3 strain decreased shoot dry weight by 48%. Abbott and Robson (1981) tested four VAM isolates on subterranean clover in two field soils. They also found that these VAM differed in their ability to promote shoot weight: \textit{G. fasciculatum} \textgreater \textit{G. monosporum} = \textit{A. laevis} > \textit{Gi. calospora} for the Merredin soil, and \textit{G. fasciculatum} \textgreater \textit{A. laevis} > \textit{G. monosporum} = \textit{Gi. calospora} for the York soil. El-Atrash et al. (1989) studied the effectiveness of five VAM isolates to promote the growth of alfalfa grown in two Syrian soils in presence of the indigenous VAM. The order of effectiveness in soil A (pH 7.86, Olsen-P 10 ppm and indigenous VAM spore count 156 per 100 cc) for the five species were: \textit{G. mosseae} \textgreater \textit{Gi. margarita} \textgreater \textit{G. fasciculatum} \textgreater \textit{Glomus} sp E3 \textgreater \textit{A. laevis} = uninoculated control. In soil B (pH 7.9, Olsen-P 11 ppm and spore 101 per 100 cc) the trend was: \textit{G. fasciculatum} \textgreater \textit{G. mosseae} \textgreater \textit{Glomus} sp. > \textit{Gi. margarita} = \textit{A. laevis} = uninoculated control. These results indicate that the effectiveness of VAM fungi may differ depending upon the soils used.

2.7.4.1 Reasons for difference in efficacy between VAM species

The ability of VAM to promote plant growth is related to many factors such as time and rate of root infection, and the development of external hyphae from the infected roots. It may also be related to the capacity of the external hyphae to absorb nutrients from soils, and the rate of the release of nutrients from the fungal hyphae into the host cells.
Unfortunately, most studies in the literature have assessed VAM using different hosts, growth conditions and unknown quantities of inoculum (Mosse 1975; Powell 1979; Bagyaraj and Sreeramulu 1982; Powell et al. 1982; Carling et al. 1979; Daniels and Menge 1981; Schenck and Smith 1982; Daft and Hogarth 1983). Nevertheless, these studies indicate that effectiveness is related to root infection levels (Daft and Nicolson 1966; Sanders et al. 1977; Abbott and Robson 1977b; Hayman and Stovold 1979; Medina et al. 1988; Hung et al. 1990).

Sanders et al. (1977) studied the effects of VAM inoculants (G. mosseae, G. macrocarpum var. geosporum Gerd. and Trappe, G. microcarpum Tul. and Tul., Gi. calospora Gerd. and Trappe) on the growth of onion. They observed that the magnitude of the growth increase was positively correlated with the rate and level of VAM infection by these different endophytes. Similarly, Medina et al. (1988) observed a significant positive correlation between mycorrhizal root colonization, expressed as either a percentage or the total root length colonized, and shoot dry weight for Macroptilium atropurpureum Urb. cv. Siratro.

Some VAM isolates produce similar levels of VAM infection in the roots but differ in their ability to stimulate host growth. Such variation could be due to either differences in the characteristics of the external hyphae such as length, spread and distribution in soil, and P uptake or to functional differences at the level of the host fungus interface.

Vesicular-arbuscular mycorrhizal fungi vary considerably in their ability to produce external hyphae (Sanders et al. 1977; Abbott and Robson 1985; Kothari et al. 1991; Sylvia 1992), but there is no clear relationship between the amount of hyphae and the growth response observed in colonized plants. Graham et al. (1982a) found that differences in effectiveness of eight Glomus isolates on citrus were a function of the amount of external hyphae (estimated by weight of soil they had bound into aggregates).
However, Abbott and Robson (1985) found no relationship between the total length of external hyphae produced by different fungal isolates and the growth of mycorrhizal subterranean clover. Jakobsen et al. (1992) did show that the efficiency of P uptake by a VA mycorrhizal fungus is strongly affected by the amount of hyphae produced, the spatial distribution of the external hyphae and the capacity for P uptake per unit length of hyphae. For example, they found that A. laevis Gerdemann and Trappe maintained a constant hyphal density up to 11 cm from the roots of subterraneum clover (Trifolium subterraneum L.) (47 days after seeding), whereas the hyphal density of Scutellospora calospora (Nicol. and Gerd.) Walker and Sanders declined exponentially with increasing distance from the roots. A Glomus sp. had an intermediate hyphal distribution pattern from the roots. The average rate of hyphal spread was 3.1 (mm d⁻¹) for A. laevis and 0.7-0.8 (mm d⁻¹) for Sc. calospora and the Glomus sp. Acaulospora laevis also produced the largest increase in P uptake and growth of subterraneum clover. The phosphorus inflow per unit mycorrhizal root length was 2.5-3 times as high with A. laevis as with the other two fungi. This study demonstrates that the effectiveness of some VAM fungi is related to external hyphal characteristics.

The ability of different VAM species to produce arbuscules, the most important structures in the transfer of nutrient between the host and the fungus, may determine the relative efficacy of different VAM to promote host growth. Clapperton and Reid (1992) observed a significant difference in the percentage of arbuscular infection in roots of timothy grass (Phleum pratense L.) and slender wheat grass [(Agropyron trachycaulum (Link) Male ] grown in soils containing different compositions of VAM species. They suggested that different VAM species might have predominated in the colonization of the two grasses, reflecting different levels of arbuscule production. Similarly, Miller et al. (1989) found that arbuscule production by G. mosseae in apple (Malus sylvestris L.) was more persistent than that by G. macrocarpum. They suggested that persistent arbuscule
production by *G. mosseae* may be related to the higher efficacy of *G. mosseae* observed on apple. More studies are needed to relate differences in the level of arbuscular infection to the efficacy of different VAM isolates to stimulate host growth.

### 2.7.4.2 Performance of VAM under varying host-environment conditions

The efficacy of a VAM may be modified if the environmental conditions are changed (Table 2.7.1). Thus efficacy is a product of the inherent attributes of a VAM species plus the growth conditions of the symbiotic partners. It is important, therefore, to assess the performance of VAM isolates under different host-environment combinations. The goal of such studies is to choose VAM species that are effective over a wide range of host-environment combinations.

Schubert and Hayman (1986) found that the efficacy of *G. macrocarpum*, *G. mosseae* and *Gi. margarita* on onion decreased when available soil P was increased (Table 2.7.1). Koomen et al. (1987) found that the performance of *G. macrocarpum* was better than *G. mosseae* on clover grown at pH 4.8. When the pH of the growth medium was increased to 6.8 *G. mosseae* performed better than *G. macrocarpum* (Table 2.7.1). Hayman and Tavares (1985) (Table 2.7.1) and Skipper and Smith (1979) reported similar results. These studies indicate that the ability of VAM species to increase host growth differs depending upon the pH of the growth medium. Bethlenfalvay et al. (1982) found that the effect of two VAM, *G. fasciculatum* and *G. mosseae*, on lentil and sorghum was different depending upon the type of soils. For example, the increase in growth of soybean inoculated with either VAM in 3 soils was: Josephine soil (pH 6.9; plant available P, 4.6 ppm) > Balcom soil (pH 8.0; plant available P, 3.3 ppm) > Corning soil (pH 6.1; plant available P, 3.5 ppm). On the other hand, *G. mosseae* increased sorghum growth seven-fold in Balcom soil compared to *G. fasciculatum*, whereas the trend was reversed when the sorghum was grown in Josephine soil. Similar results are reported by
Table 2.7.1 Growth of different host crops inoculated with VAM and grown in sterilized soils of different pH and with different levels of plant available P

<table>
<thead>
<tr>
<th>VAM Isolate</th>
<th>Inoculum</th>
<th>Host crop</th>
<th>Growth conditions</th>
<th>Increase in shoot dry wt (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phosphorus (µg g⁻¹)</td>
<td>pH</td>
<td>Crop age (week)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomus macrocarpum</td>
<td>0.3 g roots + sievings</td>
<td>Onion</td>
<td>4</td>
<td>5.2</td>
<td>0 21 383 Schubert and Hayman 1986</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>0.3 g roots + sievings</td>
<td>Onion</td>
<td>4</td>
<td>5.2</td>
<td>25 147 3183</td>
</tr>
<tr>
<td>Gigaspora margarita</td>
<td>0.3 g roots + sievings</td>
<td>Onion</td>
<td>4</td>
<td>5.2</td>
<td>50 74 605</td>
</tr>
<tr>
<td>Glomus macrocarpum</td>
<td>0.3 g roots + sievings</td>
<td>Onion</td>
<td>17</td>
<td>5.2</td>
<td>28 125 100</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>0.3 g roots + sievings</td>
<td>Onion</td>
<td>17</td>
<td>5.2</td>
<td>25 47 6</td>
</tr>
<tr>
<td>Gigaspora margarita</td>
<td>0.3 g roots + sievings</td>
<td>Onion</td>
<td>17</td>
<td>5.2</td>
<td>19 231 26</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>7 g soil culture</td>
<td>Strawberry</td>
<td>4</td>
<td>4.0</td>
<td>0 0 50 Hayman and Tavares 1985</td>
</tr>
<tr>
<td>Glomus epigaenum</td>
<td>7 g soil culture</td>
<td>Strawberry</td>
<td>4</td>
<td>4.0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Glomus clarum</td>
<td>7 g soil culture</td>
<td>Strawberry</td>
<td>4</td>
<td>4.0</td>
<td>0 150 900</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>7 g soil culture</td>
<td>Strawberry</td>
<td>4</td>
<td>5.0</td>
<td>50 50 100</td>
</tr>
<tr>
<td>Glomus epigaenum</td>
<td>7 g soil culture</td>
<td>Strawberry</td>
<td>4</td>
<td>5.0</td>
<td>0 50 100</td>
</tr>
<tr>
<td>Glomus clarum</td>
<td>7 g soil culture</td>
<td>Strawberry</td>
<td>4</td>
<td>5.0</td>
<td>0 200 900</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>7 g soil culture</td>
<td>Strawberry</td>
<td>4</td>
<td>7.0</td>
<td>0 1400 215</td>
</tr>
<tr>
<td>Glomus epigaenum</td>
<td>7 g soil culture</td>
<td>Strawberry</td>
<td>4</td>
<td>7.0</td>
<td>0 1200 2615</td>
</tr>
<tr>
<td>Glomus clarum</td>
<td>7 g soil culture</td>
<td>Strawberry</td>
<td>4</td>
<td>7.0</td>
<td>0 0 0</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>Sievings of 2 g culture</td>
<td>Clover</td>
<td>13</td>
<td>4.8</td>
<td>- - 306 Koomen et al. 1987</td>
</tr>
<tr>
<td>Glomus macrocarpum</td>
<td>Sievings of 2 g culture</td>
<td>Clover</td>
<td>13</td>
<td>4.8</td>
<td>- - 1027</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>Sievings of 2 g culture</td>
<td>Clover</td>
<td>13</td>
<td>6.8</td>
<td>- - 1078</td>
</tr>
<tr>
<td>Glomus macrocarpum</td>
<td>Sievings of 2 g culture</td>
<td>Clover</td>
<td>13</td>
<td>6.8</td>
<td>- - 494</td>
</tr>
</tbody>
</table>
other workers (Hung et al. 1990; Krishna and Dart 1984; El-Atrash et al. 1989).

However, the reasons for variation in the performance of VAM in different host-soil combinations are not well understood.

2.7.5 Growth depression of host crops by VAM isolates

Benefits of mycorrhizal infection may disappear depending upon growth conditions (Diedrichs 1983; Kiernan et al. 1983). Indeed, infection of plants by VAM has been shown to depress plant growth under certain conditions (Buwalda and Goh 1982). For example, growth depressions of mycorrhizal plants have been attributed to P toxicity in soils with high available P (Mosse 1973b). Most cases of growth depression, however, are considered to be caused by competition between plant and fungus for carbon (Buwalda and Goh 1982). This type of depression occurs whenever the loss of carbon caused by VAM infection exceeds the gain in carbon from enhanced photosynthesis. Such conditions are most likely to occur either when P is not limiting or when photosynthesis is light limited. However, even under conditions of low available P and relatively high intensity of light, mycorrhizal infection has been shown to cause transient growth depressions for up to 16 weeks (Bethlenfalvay et al. 1982). Transient growth depression of host plants due to VAM inoculation has been reported for many crops (Buwalda and Goh 1982; Allen and Allen 1988; Miller et al. 1989). Under similar growth condition, some VAM species may depress growth, whereas others may increase growth. For example, Hung et al. (1990) found that the vine length of industrial sweet potato (Ipomea batatas L.) inoculated with G. etunicatum was increased, whereas vine length of those inoculated with Sc. calospora S329 was reduced compared to the uninoculated controls. In the same study, plants inoculated with Acaulospora rugosa Morton S347 had less shoot biomass than controls, and plants inoculated with G. etunicatum S346 and A. rugosa S347 had less root biomass than controls.
A few studies have reported that some VAM fungi may act as pathogens and decrease plant growth by causing plant disease. For example, Hendrix and Csinos (1985) found that an isolate of *G. macrocarpum* induced stunting disease of tobacco (*Nicotiana tabacum* L.). Further, the degree of stunting of tobacco was related to the extent of root colonization. Modjo et al. (1987) verified the association of *G. macrocarpum* with the tobacco stunting disease in the field, and controlled the disease by soil fumigants. Hendrix et al. (1992) reported that the incidence of pathogenic *G. macrocarpum* could be reduced by either including tall fescue in the rotation or by soil fumigants.

### 2.7.6 Mycorrhizal dependency of host crops

There is no well-defined specificity in the ability of VAM to infect particular host species or varieties. Nor is there evidence for a broader specificity of the type observed in *Rhizobium*-legume cross inoculum groups (Harley and Smith 1983). Nevertheless, differences in the beneficial effects of particular host and fungus combinations have been observed. In the *Rhizobium* legume symbiosis, a particular combination of *Rhizobium* and legume crop is more effective in terms of nodulation and nitrogen fixation. In some of the mycorrhizal examples, the relative effectiveness of different host and fungus combinations may be correlated with differences in root morphology and root hair production by the different hosts inoculated with VAM (Harley and Smith 1983; Kothari et al. 1990b). Thus the mycorrhizal plants show differences in root morphology as a result of interaction with VAM. On the other hand, mycorrhizal plants may also be categorized on the magnitude of their growth response to a particular VAM.

The term 'mycorrhizal dependency' (Gerdemann 1975) was used to qualify the growth response of a host to VAM infection. Mycorrhizal dependency was defined as the degree to which a plant is dependent on the mycorrhizal conditions to produce its
maximum growth at a given level of soil fertility. Menge et al. (1978b) calculated mycorrhizal dependency as the dry weight of the mycorrhizal plant expressed as a percentage of the dry weight of the non-mycorrhizal plant grown under the same experimental conditions. Many authors have determined the mycorrhizal dependency of several plant species by growing VAM inoculated and control plants at a single solution P concentration (Nemec 1978; Azcon and Ocampo 1981; Plenchette et al. 1983; Pope et al. 1983; Saif 1987; Hetrick et al. 1988b). However, mycorrhizal dependency of species may change depending upon soil solution P concentration (Linderman and Hendrix 1982) or changes in other soil properties such as pH. For example, Habte and Manjunath (1991) evaluated the mycorrhizal dependency of several plant species including black mustard (Brassica nigra), leucaena (Leucaena sp.) and sesbania (Sesbania sp.) as indicator hosts across a gradient of established soil solution P concentrations. They observed that the mycorrhizal dependency of all the species tested except leucaena (Leucaena retusa L.) was similar at a soil solution P concentration of 0.002 mg L⁻¹. However, the plant species separated into distinct categories of VAM dependency as the soil P concentration was increased from 0.002 to 0.02 mg L⁻¹. Unfortunately, Habte and Manjunath (1991) used only one VAM species to determine the categories of VAM dependency. Dependency of host species on VAM may also differ depending upon the VAM species assessed.

2.7.7 Interaction between VAM isolates

Field plants are frequently infected by more than one VAM species, and any introduced VAM will interact with the indigenous mycorrhizal flora. The nature of this interaction may be competitive, synergistic or neutral for different VAM species have been evaluated mostly in growth chamber studies. The evidence of competition between VAM species has been obtained by comparing growth of host plants inoculated with VAM species alone and in combination (Table 2.7.2). For example, Koomen et al. (1987)
Table 2.7.2 Competition between VAM species in terms of host growth, VAM infection and spore production in the root region of host plants inoculated with a single VAM or a mixture of VAM species

<table>
<thead>
<tr>
<th>VAM inoculant</th>
<th>Host crop</th>
<th>Shoot dry weight (mg)</th>
<th>VAM infection (%)</th>
<th>Number of spore g⁻¹</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td><em>Trifolium repens</em></td>
<td>93</td>
<td>0</td>
<td>0</td>
<td>Koomen et al. 1987</td>
</tr>
<tr>
<td><em>Glomus mosseae</em></td>
<td><em>Trifolium repens</em></td>
<td>1096</td>
<td>85</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td><em>Glomus E3</em></td>
<td><em>Trifolium repens</em></td>
<td>188</td>
<td>82</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td><em>Glomus mosseae</em> + <em>Glomus E3</em></td>
<td><em>Trifolium repens</em></td>
<td>616</td>
<td>90</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><em>Glomus mosseae</em></td>
<td><em>Allium porum</em></td>
<td>1830</td>
<td>-</td>
<td>-</td>
<td>Hepper et al. 1988</td>
</tr>
<tr>
<td><em>Glomus caledonium</em></td>
<td><em>Allium porum</em></td>
<td>1640</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Glomus mosseae</em> + <em>Glomus caledonium</em></td>
<td><em>Allium porum</em></td>
<td>3360</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>Glomus caledonium</em></td>
<td><em>Zea mays</em></td>
<td>16570</td>
<td>87</td>
<td>107610</td>
<td>Daft and Hogarth 1983b</td>
</tr>
<tr>
<td><em>Glomus clarum</em></td>
<td><em>Zea mays</em></td>
<td>16800</td>
<td>70</td>
<td>186708</td>
<td></td>
</tr>
<tr>
<td><em>Glomus caledonium</em> + <em>Glomus clarum</em></td>
<td><em>Zea mays</em></td>
<td>17320</td>
<td>80</td>
<td>14408</td>
<td></td>
</tr>
</tbody>
</table>

*a* *Glomus caledonium* inoculum in co-inoculated treatment was uniformly distributed throughout the growth substrate, whereas *Glomus mosseae* inoculum was put 15 mm from the surface of the pot where leek seedlings were transplanted.

*b* The spore numbers were reported per plant basis.
studied the effect of mycorrhiza inocula containing one to four species of *Glomus* on white clover and strawberry plants grown at two pH levels in a low phosphate, sterilized soil (Table 2.7.2). They found that the individual inoculum (2 g of soil with VAM propagule mixture containing > 100 spores g⁻¹) of *G. mosseae* or *Glomus* sp. E3 increased shoot dry matter by 1075 or 102 % over the uninoculated control (Table 2.7.2). However, co-inoculation with an equal mixture of *G. mosseae* and *Glomus* sp. "E3" inoculum decreased the efficacy of *G. mosseae* on white clover. On the other hand Hepper et al. (1988) observed a synergistic effect due to co-inoculation of leek with *G. mosseae* and *G. caledonium* (Table 2.7.2). Miller et al. (1989) studied the effect of inoculation of apple seedlings with *G. mosseae* and *G. macrocarpum* alone and in combination. The two species increased growth rate of the apple seedlings over the uninoculated control, but the *G. mosseae* inoculant was more effective than *G. macrocarpum*. However, when *G. mosseae* was co-inoculated with *G. macrocarpum*, the effectiveness of *G. mosseae* was not changed. Similarly Daft and Hogarth (1983) found no difference in shoot dry weight of 18 week-old maize (Table 2.7.2) and onion inoculated with 60 spores of *G. caledonium* and *Glomus clarum* Nicolson and Schenck or an equal mixtures of spores of these species. These studies show that the presence of more than one species in the VAM inoculum may or may not modify the effect produced by a single VAM on a host plant.

Precise methods for evaluating competition between VAM species should be based on the relative proportion of infected root area occupied by individual VAM. Methods to distinguish anatomical structures produced by different VAM inside roots are relatively scarce (Abbott and Robson 1978; Wilson 1984). Wilson (1984) studied competition between *Gi. decipiens*, *G. fasciculatum* and *Glomus tenue* (Greenall) Hall for infection of clover (*Trifolium subterraneum* L.). In various combinations, *Gi. decipiens* appeared to be the more aggressive VAM infecting the host. There was no interaction between *G.*
fasciculatum and G. tenue. In general, the level of infection in the host root due to inoculation with a mixture of high and low infective species was similar to that produced by inoculation with the more infective species alone. Hepper et al. (1988) evaluated the competitive ability of three species by analyzing the infected roots for the presence of diagnostic fungal enzymes. Vesicular-arbuscular mycorrhizal spore production in the rhizosphere of plants also has been used as a measure of competitive ability of the individual VAM species (Koomen et al. 1987; Daft and Hogarth 1983). For example, Koomen et al. (1987) found that four VAM species produced large numbers of spores on the strawberry plants when inoculated alone (Table 2.7.2). Co-inoculation of these species in different mixtures reduced spore production by individual species. However, G. clarum maintained its higher sporulation ability in all different combinations, reflecting its higher competitive ability. Similarly, Daft and Hogarth (1983) found G. clarum to be a highly competitive VAM among the isolates they tested (Table 2.7.2).

2.7.8 Interaction between host genotypes and VAM

In recent years, many experiments have been conducted to study the response of various crop genotypes to VAM. The results of these experiments suggest a possible interaction between host genotypes and VAM at the molecular level. For example, Hall (1978b) found that a maize variety which had a rapid growth rate, extensive root system and low tissue phosphate concentrations did not respond to mycorrhizal infection, whereas maize cv. 415 and cv. Golden Cross Bantam, which had slower growth rates, less root development and higher tissue phosphate concentrations were responsive to infection (Table 2.7.3). Plant response could not be attributed to differences in the percentage of VAM infection of the root system measured at the final harvest. Similar results were obtained by Lambert et al. (1980) using clones of alfalfa, by Menge et al.
Table 2.7.3. Differential response of plant genotypes to VAM inoculation as determined by root infection and shoot growth

<table>
<thead>
<tr>
<th>Crop</th>
<th>Genotypes</th>
<th>VAM infection (%)</th>
<th>Shoot dry weight (g)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>Inoculation</td>
</tr>
<tr>
<td>Zea mays</td>
<td>P × 610</td>
<td>28</td>
<td>12.02</td>
<td>11.73</td>
</tr>
<tr>
<td></td>
<td>W 415</td>
<td>30</td>
<td>5.08</td>
<td>10.19</td>
</tr>
<tr>
<td></td>
<td>Golden Cross Bantam</td>
<td>33</td>
<td>5.90</td>
<td>8.63</td>
</tr>
<tr>
<td>Arachis hypogaea</td>
<td>TMV 2</td>
<td>74</td>
<td>0.57</td>
<td>2.65</td>
</tr>
<tr>
<td></td>
<td>NCAC 17142</td>
<td>65</td>
<td>0.90</td>
<td>1.97</td>
</tr>
<tr>
<td></td>
<td>ICG 4504</td>
<td>65</td>
<td>1.17</td>
<td>2.29</td>
</tr>
<tr>
<td>Cicer aeratinum</td>
<td>Annigeri</td>
<td>42</td>
<td>0.56</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>G 130</td>
<td>39</td>
<td>0.51</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>K 850</td>
<td>47</td>
<td>0.71</td>
<td>1.04</td>
</tr>
<tr>
<td>Medicago sativa</td>
<td>Nod−Fix− line</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Nod+Fix− line</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Nod+Fix+ line</td>
<td>47</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
(1978b) in citrus cultivars, by Nemec (1978) with citrus rootstock, by Rao et al. (1990) in peanut genotypes, and by Daft (1991) (Table 2.7.3) in chickpea (Cicer arietinum L.) and peanut genotypes.

Thus host genotype may be an important determinant in mycorrhizal response, as it is with the *Rhizobium*-legume symbiosis. Duc et al. (1989) reported that genetic resistance to VAM formation has been obtained in spontaneous or chemically induced mutants of mycorrhiza-forming species garden pea (Pisum sativum L.) and faba bean (Vicia faba L.). The eight mutants, termed myc- were characterized by aborted infections limited to one or two host cells. Expression of the myc- character was associated with that of the nod- character in both legumes. Preliminary analysis of the genetic behavior of the myc- mutants in diallel crosses showed that at least three genes are involved in VAM infection. Bradbury et al. (1991) also found that seedlings of a non-nodulating alfalfa genotype (nod- fix-) and an ineffectively nodulating alfalfa genotype (nod+fix-) exhibited resistance to VAM colonization when grown in pot cultures inoculated with either *G. versiforme* or *G. intraradices*. The fungus failed to form any internal structures such as arbuscules and vesicles in the roots of nod-fix- alfalfa genotype, whereas wild type (nod+fix+) alfalfa seedlings were colonized normally (Table 2.7.3).

2.8 General conclusions

Vesicular-arbuscular mycorrhizae are ubiquitous in plants and soils of both natural ecosystems and man made agroecosystems. Although VAM infection occurs in different plant habitats, the direct evidence regarding benefits of VAM to plant growth in natural ecosystems has not been obtained. In recent years much emphasis has been attached to reclamation of damaged natural plant habitats and revegetation of areas where natural vegetation has been completely eliminated by human actions. In these cases, knowledge of VAM population dynamics and activity will be extremely useful. In fact, experimental
evidence suggest that VAM symbiosis improves seedling establishment in newly restored plant habitats.

The conversion of land with native vegetation to agricultural production probably disturbs the fine balance that exists between VAM and plant species. In this process some beneficial VAM species may be eliminated from the habitat. Agricultural practices such as fertilizer application, crop rotation, fungicide and pesticide applications, and tillage operations have a distinct impact on VAM dynamics and activity in agricultural soils and crops. The impact of these factors are evaluated in either field survey studies or in growth chamber using either mixed inocula or a monospecific VAM culture. These VAM isolates are usually obtained from local agricultural field soils.

Inocula of VAM isolates obtained from agricultural field soils increase the growth and yield of crops in growth chamber and field studies. In a few cases VAM inoculants reduced plant growth. Furthermore, the positive growth response of crops to VAM inoculation may differ depending upon host-fungus-environment combinations. Competition between VAM species may influence the efficacy of a VAM inoculum. Nevertheless, there is a potential to use VAM as "biofertilizers" in commercial agriculture of a specific region. Additional studies are needed on dynamics and activity of VAM in agricultural soils and crops to provide knowledge for effective management of VAM during production of crops in different agroecosystems.
evidence suggest that VAM symbiosis improves seedling establishment in newly restored plant habitats.

The conversion of land with native vegetation to agricultural production probably disturbs the fine balance that exists between VAM and plant species. In this process some beneficial VAM species may be eliminated from the habitat. Agricultural practices such as fertilizer application, crop rotation, fungicide and pesticide applications, and tillage operations have a distinct impact on VAM dynamics and activity in agricultural soils and crops. The impact of these factors are evaluated in either field survey studies or in growth chamber using either mixed inocula or a monospecific VAM culture. These VAM isolates are usually obtained from local agricultural field soils.

Inocula of VAM isolates obtained from agricultural field soils increase the growth and yield of crops in growth chamber and field studies. In a few cases VAM inoculants reduced plant growth. Furthermore, the positive growth response of crops to VAM inoculation may differ depending upon host-fungus-environment combinations. Competition between VAM species may influence the efficacy of a VAM inoculum. Nevertheless, there is a potential to use VAM as "biofertilizers" in commercial agriculture of a specific region. Additional studies are needed on dynamics and activity of VAM in agricultural soils and crops to provide knowledge for effective management of VAM during production of crops in different agroecosystems.
3 EXPERIMENTAL

3.1 Occurrence and isolation of vesicular-arbuscular mycorrhizae in cropped field soils of Saskatchewan

3.1.1 Introduction

Vesicular-arbuscular mycorrhizae (VAM) are ubiquitous in soils, and play an important role in plant growth and development. However, the VAM community and VAM infection of plant roots may vary greatly in different soils (Abbott and Robson 1977a; Schenck and Smith 1981; Jakobsen and Nielsen 1983; Hall and Abott 1984; Porter et al. 1987) and under different cropping practices (Abbott and Robson 1991a; Johnson et al. 1991a). Interest in VAM has focussed on: (i) the effectiveness of various VAM species to enhance plant growth (Mosse 1972; Powell 1982; Jensen 1984a), (ii) the influence of VAM on soil aggregate stability (Tisdall and Oades 1979; Miller and Jastrow 1992), and (iii) the use of VAM inoculants to increase plant growth under field conditions in the presence of indigenous VAM (Mosse and Hayman 1971; Owusu-Bennoah and Mosse 1979; Gianinazzi et al. 1989). The potential for increasing crop growth by effective management of VAM strains reinforces the need to determine VAM infection of crop plants and the species composition of the VAM community indigenous to cultivated soils of a particular location. Such studies are also necessary for isolating indigenous VAM strains to be assessed in local crop production. Vesicular-arbuscular mycorrhizal spores are abundant and two VAM species have been reported for Saskatchewan soils (Bakerspigel 1956; Kucey 1980). However, no systematic studies have determined the types of VAM in Saskatchewan soils. Here I report the occurrence and isolation of different VAM species in field cropped with wheat and lentil in Saskatchewan.
3.1.2 Materials and Methods

3.1.2.1 Field study sites

Infection of wheat (*Triticum aestivum* L. cv. Katepwa) and lentil (*Lens esculenta* L. cv. Eston) was monitored during the 1988 and 1989 growing seasons at 11 study sites (Table 3.1.2.1). The number of VAM spores at 10 sites was determined in 1988. These sites are representative of the major soil zones of Saskatchewan, and are located along an environmental gradient of increasing soil moisture and decreasing temperature from southwest to northeast (Fig. 3.1.2.1). All sites had been cultivated for more than 50 years except the Medstead site which was cleared of forest vegetation in 1982. Each field site had been cropped to wheat for at least three years prior to 1988. In 1988, the wheat and lentil crops received 10 and 22.5 kg P ha⁻¹, respectively, at the time of seeding.

3.1.2.2 Determination of VAM spore numbers

Soil samples (20 to 30 cores of 2.5 cm diam. × 15-20 cm length) were collected from the wheat field at each 1988 study site at the time of seeding, crushed and bulked together. Four 100-g subsamples were analyzed for VAM spore numbers. Spores were obtained by wet sieving (300-53 μm opening diameter) and decanting (Gerdemann and Nicolson 1963). These spores were cleared of organic detritus and soil particles by sucrose density gradient centrifugation and washing (Mertz et al. 1979), and a spore suspension made in distilled water. This spore suspension was distributed on two 0.8-μm gridded millipore filters and spore numbers were counted with a stereomicroscope (47×). During counting, morphologically similar spores were separated into groups, mounted, and identified (see below).
3.1.2.1 Location of VAM survey sites in Saskatchewan.
Note: DB=Dark Brown, BL=Black, GR=Gray.
Table 3.1.2.1 Soil and crop characteristics of VAM survey sites

<table>
<thead>
<tr>
<th>Field site</th>
<th>Soil type</th>
<th>Soil characteristics (1988)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Details of VAM survey</th>
<th>VAM spores&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>OM (%)</td>
<td>NO₃-N (μg g⁻¹)</td>
<td>P</td>
</tr>
<tr>
<td>Kindersley</td>
<td>7.0</td>
<td>3.0</td>
<td>30</td>
<td>18</td>
</tr>
<tr>
<td>Lucky lake</td>
<td>7.0</td>
<td>2.5</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Sceptre</td>
<td>7.6</td>
<td>2.4</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Brown Chernozems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outlook</td>
<td>7.6</td>
<td>2.1</td>
<td>43</td>
<td>10</td>
</tr>
<tr>
<td>Semans</td>
<td>7.3</td>
<td>3.9</td>
<td>12</td>
<td>35</td>
</tr>
<tr>
<td>Watrous</td>
<td>7.7</td>
<td>3.2</td>
<td>78</td>
<td>34</td>
</tr>
<tr>
<td>Dark Brown Chernozems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Englefeld</td>
<td>7.3</td>
<td>9.2</td>
<td>130</td>
<td>60</td>
</tr>
<tr>
<td>Leroy</td>
<td>7.4</td>
<td>8.0</td>
<td>132</td>
<td>57</td>
</tr>
<tr>
<td>Glenavon</td>
<td>7.4</td>
<td>4.5</td>
<td>64</td>
<td>45</td>
</tr>
<tr>
<td>Gray Luvisols</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenen</td>
<td>7.5</td>
<td>5.9</td>
<td>30</td>
<td>24</td>
</tr>
<tr>
<td>Medstead</td>
<td>7.6</td>
<td>8.5</td>
<td>84</td>
<td>18</td>
</tr>
</tbody>
</table>

<sup>a</sup>Determined by the Saskatchewan soil testing laboratory; P, plant available.

<sup>b</sup>Wheat and lentil were grown in adjacent fields at each site except Semans, Watrous and Leroy where the fields were 1000, 500 and 200 meters apart, respectively.

<sup>c</sup>VAM spores enumerated from soils collected at seeding of 1988 wheat fields in 1988 and isolated from soils collected at harvest of wheat in 1989.

Note: +, VAM studied; -, VAM not studied.
3.1.2.3  VAM infection of wheat and lentil

Plants were sampled throughout the growing season at times coinciding with various physiological crop stages. Wheat was sampled at tillering, panicle emergence and maturity. Lentil was sampled at initiation of flowering, pod formation and seed maturity. Crops were sampled four times at the Englefeld and Leroy sites during 1988. Twenty to thirty plant roots were randomly sampled from an area of 200 m² at each study site. The roots and surrounding soil of individual plant were excavated to a depth of 15-20 cm. The roots were washed free of soil, samples from each site were combined and stored at -30°C for 2-3 months before estimation of VAM infection. Soil samples (2.5 cm diam. x 15-20 cm length cores) were collected adjacent to each plant sampled (at the first sampling interval), bulked together, air dried and the composite samples used to determine soil characteristics for each field site (Table 3.1.2.1).

The composite root sample from each site was divided into three subsamples. The 1988 samples were cleared, stained (Phillips and Hayman 1970) and 50 randomly selected root segments (1 cm length) were mounted using Permount fixative SO-P-15B (Fisher Scientific Company, Chemical Division, Fair Lawn, New Jersey 07410 USA) on large microscope slides (75 x 38 mm). Each root segment was examined using a stereomicroscope (47x) and the length of root infected with VAM estimated. This slide method was time consuming and the infection values were not significantly different than those obtained by the grid line intersect method (Giovannetti and Mosse 1980). Thus, the 1989 root samples were evaluated by the grid-line intersect method. Root samples were cut into 1-cm lengths, mixed, a 3-g sample (fresh wt.) stained and assessed for percentage of VAM infected root length. Different types of vesicle structures in the wheat roots collected at different field sites were also noted and photomicrographs taken.
3.1.2.4 Isolation and identification of VAM

Soils from wheat fields at six different sites were used for isolation of VAM species (Table 3.1.2.1). Soil samples were collected from the wheat fields at harvest in August of 1989. Twenty to thirty soil cores (1.5 cm diam. × 15-20 cm length) were collected from an ca. 50 m² area at each site and mixed thoroughly. Soil samples were stored at 7°C for two months before enrichment of VAM present in these soils was carried out. Indigenous VAM propagules (spores, hyphae and infected root pieces) were obtained by sieving 100-g soil subsamples through a 500 μm sieve and collecting the materials retained on a 53 μm sieve. These indigenous VAM mixtures from each soil were propagated in a growth chamber using maize as the host crop.

The Outlook soil (Table 3.1.2.1) was mixed with a coarse sand (silica sand grade #7 Target Product Ltd., Calgary - AB, Canada) and 6 kg of this soil:sand mixture potted in plastic pots (22 cm diam. × 21 cm long); 300 mL of a modified Hoagland nutrient solution [μg mL⁻¹: KNO₃, 540; KH₂PO₄, 133; K₂SO₄, 176; CaSO₄ 2H₂O, 500; MgSO₄ 7H₂O, 103; and micronutrients at one-third of normal levels (Hoagland and Arnon 1938)] were added to compensate the nutrients due to dilution of the original soil nutrients. Pots were autoclaved twice for 1 h at a 24 h interval to eliminate indigenous VAM. The autoclaved soil:sand mix had a pH of 7.6 and contained (μg g⁻¹) NO₃-N, 30; P, 10; K, 320; SO₄-S, 15.6; Cu, 0.5; Fe, 7.5; Zn, 1.36; and Mn, 75.

Maize seeds were surface sterilized by treating with 70% (v/v) ethyl alcohol for 1 min, 1.2% (w/v) sodium hypochlorite for 5 min, and washed 8 times with sterile distilled water. Seeds were aseptically germinated on Trypticase soy agar (0.3% Trypticase soy broth, 1.5% agar). Aluminum foil funnels were placed at the center of each pot and soil sievings (containing indigenous VAM mixtures of a given soil) carefully placed in the necks (diam. 1.5 cm) of each funnel (diam. 4.5 cm). Two maize seedlings were planted
to each funnel to allow the growing roots to be infected by VAM. Control pots were planted with maize seedlings to check the effectiveness of sterilization procedure in eliminating the indigenous VAM from the soil:sand mixture. Pots were placed in a growth chamber (photosynthetic irradiance of 340-400 μE m⁻² s⁻¹ with a 16 h day (26°C) : 8 h night (20°C) cycle. One hundred mL of a modified Hoagland solution (above, minus P) was added to each pot every week during the first eight weeks of plant growth. Soil:sand mix was maintained at 70% moisture holding capacity (ca. - 60 kPa soil water potential) and watered daily with sterile distilled water to constant weight.

Plants were grown for 90 days. At harvest, maize shoots were chopped off and the soil:sand mix containing roots and VAM propagules was air dried for one week at 25°C. These root-soil mixtures (enriched for VAM propagules from selected field soils) were thoroughly mixed. Vesicular-arbuscular mycorrhizal spores were recovered from 100-g samples of root-soil mixtures using the wet sieving (500-53 μm opening diameter) and density gradient centrifugation method (Daniels and Skipper 1982) (Fig. 3.1.2.2). Spores were washed in tap water and a suspension made up to 100 mL. Spores were observed under a stereomicroscope (47x) and separated into different groups based on their morphology, color and type of hyphal attachment.

A minimum of 30 spores of each putative VAM species was permanently mounted on microscope slides: 10 unbroken spores in Polyvinyl alcohol-Lactic acid-Glycerol (PVLG); 10 spores broken in PVLG; 10 spores broken in PVLG + Melzer's reagent (Schenck and Perez 1988). Detailed spore characteristics such as shape, size, color, appearance of cytoplasm, wall structure, and hyphal attachment to spore wall were recorded. Photomicrographs of spores in PVLG and PVLG + Melzer's reagent were made. Spores were identified using taxonomic key described by Schenck and Perez (1988) and classified into the described species (Schenck and Perez 1988; Morton 1988). Cultures of four isolates are deposited in the INVAM, West Virginia University.
3.1.2.2 Mixture of VAM spores collected from maize rhizosphere (A) before and (B) after sucrose density gradient (20% and 60%) centrifugation.
3.1.2.5 Occurrence of VAM species in different soils

Aliquots (20 mL) of spore suspensions collected from root-soil mix were uniformly spread over a 45-mm gridded millipore filter (0.8 μm) and examined under a stereomicroscope (47×). The presence or absence of a particular VAM species in the six original field soils was based on these observations.

3.1.3 Results

3.1.3.1 VAM spore numbers

The number of VAM spores in the 1988 field soils at seeding ranged from 78-272 per 100 g soil (Table 3.1.3.1). Brown Chernozem soils generally contained fewer VAM spores than other soils. Although there was no apparent relationship between spore numbers and soil characteristics, it was noted that the Englefeld and Leroy soils which had high NO₃-N and P levels (Table 3.1.2.1) contained fewer spores than other soils (Table 3.1.3.1). The spores recovered from field soils were morphologically similar to those obtained after enrichment of indigenous VAM mixtures on maize (see below). In fact, except for Gi. decipiens, all species detected in the maize rhizosphere (i.e., enrichment) were detected in field-collected spores.

3.1.3.2 VAM infection in wheat and lentil

Vesicular-arbuscular mycorrhizae infected the roots of wheat and lentil of all study sites, during 1988 and 1989 (Figs. 3.1.3.1 and 3.1.3.2). The percentage of VAM infected wheat roots was different at each field site, but there was no definite trend in VAM infection of wheat between the four soil zones. Generally, wheat crops grown in the Dark Brown or Gray soils exhibited the highest levels of VAM infection. The low percentage of VAM infected wheat roots at all the Brown soil field sites might be related to low moisture levels at these sites (Table 3.1.3.2 and Fig. 3.1.3.1). The variation in
Table 3.1.3.1 Vesicular-arbuscular mycorrhizal spore numbers in Saskatchewan field soils at seeding of 1988 wheat crop

<table>
<thead>
<tr>
<th>Soil zone</th>
<th>Field site</th>
<th>No. of spore (per 100 g soil)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown</td>
<td>Kindersley</td>
<td>121 ± 30.7</td>
</tr>
<tr>
<td></td>
<td>Lucky lake</td>
<td>78 ± 6.9</td>
</tr>
<tr>
<td></td>
<td>Sceptre</td>
<td>128 ± 36.1</td>
</tr>
<tr>
<td>Dark Brown</td>
<td>Outlook</td>
<td>118 ± 11.7</td>
</tr>
<tr>
<td></td>
<td>Semans</td>
<td>256 ± 86.9</td>
</tr>
<tr>
<td></td>
<td>Watrous</td>
<td>272 ± 36.6</td>
</tr>
<tr>
<td>Black</td>
<td>Englefeld</td>
<td>150 ± 29.1</td>
</tr>
<tr>
<td></td>
<td>Leroy</td>
<td>125 ± 7.8</td>
</tr>
<tr>
<td></td>
<td>Glenavon</td>
<td>262 ± 59.5</td>
</tr>
<tr>
<td>Gray Luvisols</td>
<td>Stenen</td>
<td>246 ± 75.3</td>
</tr>
</tbody>
</table>

$^a$Oven dry basis.

Note: ±, standard deviation of four replicate samples.
Figure 3.1.3.1 Vesicular-arbuscular mycorrhizal infection at different growth stages of wheat (●) and lentil (○) grown during 1988 and 1989 in the Brown and Dark Brown soil zones. Infection values are means of 3 subsamples ± S.D.
Figure 3.1.3.2 Vesicular-arbuscular mycorrhizal infection at different growth stages of wheat (●) and lentil (□) grown during 1988 and 1989 in the Black and Gray soil zones. Infection values are means of 3 subsamples ± S.D.
Table 3.1.3.2 Available soil moisture at seeding and precipitation during the 1988 growing season at Saskatchewan field sites

<table>
<thead>
<tr>
<th>Soil zone</th>
<th>Field site</th>
<th>Soil moisture to 0-60 cm (cm)</th>
<th>Precipitation (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown</td>
<td>Kindersley</td>
<td>10.0</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>Lucky lake</td>
<td>9.0</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>Sceptre</td>
<td>1.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Dark Brown</td>
<td>Outlook</td>
<td>Unlimited</td>
<td>Irrigated</td>
</tr>
<tr>
<td></td>
<td>Semans</td>
<td>25.7</td>
<td>15.0</td>
</tr>
<tr>
<td></td>
<td>Watrous</td>
<td>18.0</td>
<td>16.8</td>
</tr>
<tr>
<td>Black</td>
<td>Englefeld</td>
<td>13.0</td>
<td>27.5</td>
</tr>
<tr>
<td></td>
<td>Leroy</td>
<td>13.0</td>
<td>22.0</td>
</tr>
<tr>
<td></td>
<td>Glenavon</td>
<td>18.0</td>
<td>19.4</td>
</tr>
<tr>
<td>Gray Luvisols</td>
<td>Stenen</td>
<td>20.0</td>
<td>15.5</td>
</tr>
</tbody>
</table>
VAM infection in wheat at different sites was not significantly correlated to organic matter, NO₃-N or plant available P of soils at these sites. Lentil roots exhibited rapid and extensive infection by indigenous VAM at all Dark Brown, Black and Gray field sites where lentil was grown (Figs. 3.1.3.1 and 3.1.3.2). The apparent rate and level of VAM infection in lentil was greater than for wheat grown on adjacent fields. Although the percentage of VAM infected lentil roots grown at each field site was different, the differences were not as marked as for wheat. Based on microscopy of root segments it appeared that lentil roots contained more arbuscules and vesicles than wheat roots (Fig. 3.1.3.3). However, the shape of vesicles observed inside wheat roots varied at different field sites (Fig. 3.1.3.4). The VAM infection levels for wheat and lentil roots for the 1989 season were similar to the levels observed for these sites in 1988 (Figs. 3.1.3.1 and 3.1.3.2).

3.1.3.3 Isolation and identification of VAM

Seven VAM strains representing the genera Acaulospora, Gigaspora and Glomus were isolated from wheat field soils collected at six sites in Saskatchewan (Table 3.1.3.3). These included Acaulospora denticulata Sieverding and Toro, Gigaspora decipiens Hall and Abbott, Glomus fasciculatum Walker and Koske, G. geosporum Nicolson and Gerdemann, G. macrocarpum Tul. and Tul. (Berch and Fortin), G. mosseae (Nicolson and Gerdemann) Gerdemann and Trappe, and G. versiforme (Karsten) Berch (Fig. 3.1.3.5).

3.1.3.4 Distribution of VAM species in soils

Glomus species were the most common VAM detected in the different Saskatchewan soils with G. geosporum and G. mosseae present at all sites (Table 3.1.3.3). Acaulospora and Gigaspora spores also were frequently observed in the
Figure 3.1.3.3 Typical root segments of 50 day old field grown (Outlook site) lentil (A) and (B) containing abundant arbuscules (ar) and vesicles (ve) and wheat (C and D) containing few arbuscules and vesicles. Bar markers = 180 μm.
Figure 3.1.3.4 Different types of vesicle structures (arrows) observed in wheat roots collected at different field sites: (A and B) Detected at Outlook, Semans and Medstead. (C) Detected at Semans and Medstead. (D) Detected at Semans only. (E) Detected at Outlook and Medstead. (F) Detected at Medstead only.

Bar markers = 63 μm.
Figure 3.1.3.5 Spores of vesicular-arbuscular mycorrhizal fungi indigenous to Saskatchewan field soils. Representative spores were mounted in polyvinyl alcohol-lactic acid-glycerol and photographed using differential interference contrast microscopy, except (C) which was unmounted and photographed using stereomicroscopy. (A) *Acaulospora denticulata* Sieverding and Toro; arrows show four distinct spore walls. (B) Close-up of *Acaulospora denticulata* spore showing characteristic polygon segments (arrow) of the outer wall. (C) *Gigaspora decipiens* Hall and Abbott; arrow shows bulbous suspensor. (D) Squashed *Gigaspora decipiens* spores showing distinct spore walls (arrows). (E) *Glomus fasciculatum* Walker and Koske; loose cluster of spores. (F) *Glomus fasciculatum* spores showing attachment (arrow) of hyphae to spore wall. (G) Close-up of *Glomus fasciculatum* spore showing ingrowths (arrows) from wall. (H) *Glomus geosporum* Nicolson and Gerdemann; arrow shows thick hyphal wall at the point of attachment to the spore. (I) Squashed *Glomus geosporum* spore showing spore wall details (arrows). (J) Intact spores of *Glomus macrocarpum* (Tul. and Tul.) Berch and Fortin. (K) Squashed *Glomus macrocarpum* spore showing spore walls (arrows). (L) *Glomus mosseae* (Nicolson and Gerdemann) Gerdemann and Trappe; arrow shows characteristic funnel shape of hyphal attachment. (M) Close-up of squashed *Glomus mosseae* spore showing spore walls (arrows). (N) A portion of a *Glomus versiforme* (Karsten) Berch sporocarp. (O) Individual *Glomus versiforme* spore showing characteristic spore walls (arrows). All bar markers = 65 μm.
Table 3.1.3.3. Vesicular-arbuscular mycorrhizal spores isolated from the soil: sand mix used to grow maize inoculated with VAM spore mixtures indigenous to field soils

<table>
<thead>
<tr>
<th>VAM spores</th>
<th>Lab Isolate No.</th>
<th>INVAM No.</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Outlook</td>
</tr>
<tr>
<td>Acaulospora denticulata</td>
<td>NT1</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Gigaspora decipiens</td>
<td>NT2</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Glomus fasciculatum</td>
<td>NT3</td>
<td>SA 104</td>
<td>+</td>
</tr>
<tr>
<td>Glomus geosporum</td>
<td>NT4</td>
<td>SA 101</td>
<td>+</td>
</tr>
<tr>
<td>Glomus macrocarpum</td>
<td>NT5</td>
<td>SA 102</td>
<td>+</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>NT6</td>
<td>SA 103</td>
<td>+</td>
</tr>
<tr>
<td>Glomus versiforme</td>
<td>NT7</td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

*a* Indigenous VAM propagule mixtures (spores, hyphae and infected root pieces) were obtained from appropriate soils by wet sieving (500-53 μm) 100-g sample; the entire mixture was used to inoculate maize seedlings which were grown in a sterilized soil: sand mix for 90 days.

*b* INVAM No. assigned by Dr. J. B. Morton, Curator, INVAM culture collection and corresponds to Voucher No. (slide) as follows: SA 101 = S1718; SA 102 = S1729; SA 103 = S1720; SA 105 = S1721.

Note: +, present; -, absent.
different soils, but they were apparently from only one species. The Glenavon soil contained the least number of VAM species, whereas the Outlook and Semans soils contained the widest variety of VAM species (Table 3.1.3.3). There was no apparent relationships with the measured soil properties (i.e., pH, organic matter, NO$_3$-N and plant available P) and the occurrence of VAM species at a particular site.

3.1.4 Discussion

Vesicular-arbuscular mycorrhizal spores were abundant and readily detected in the Saskatchewan soils studied. These soils represent four major soil zones of Saskatchewan and exhibit different physico-chemical (Anderson et al. 1974) and biological characteristics (Lawrence and Germida 1988). The number of spores in these agricultural soils was similar to that detected in agricultural soils by other workers (Abbott and Robson 1977a; Hayman and Stovold 1979; Malibari et al. 1988). In general, Brown Chernozems contained fewer spores than other soils which might be a reflection of the drought condition (i.e., less crop growth) typical for the Brown soils. The VAM in all soils readily infected wheat and lentil, but the VAM infection pattern was different for these two crops. Three phases of VAM infection during the growth of field grown cereal, legume and vegetable crops have been reported by other workers (Sutton 1973; Saif 1977). I observed that VAM infection in wheat became well established by the tillering stage, increased until panicle emergence, and then tended to decline. In contrast, lentil exhibited an initial rapid infection until the flowering stage which was followed by a plateau until maturity.

Generally, lentil exhibited higher percentage of VAM infected roots compared to wheat. Jakobsen and Nielsen (1983) observed that when root density of pea and spring wheat were compared, the absolute root length of pea infected with VAM did not differ from that of spring wheat. I did not determine total root length of the two crops in this
survey study, and this comparison can not be made. Growth chamber studies showed that although the percentage of VAM infected lentil roots was higher than wheat, the actual total VAM infected root length was greatest for wheat (F. Walley, personal communication). However, this difference may not reflect a benefit obtained by the two crops from VAM infection under field conditions. I observed a greater production of arbuscules and vesicles in field grown lentil roots than in wheat roots. This difference in the infection morphology in the two crop roots might explain the generally held view that crops with coarse roots (e.g., legumes) gain more from VAM compared to those with fine roots (e.g., wheat). Alternatively, this difference in the infection morphology was due to different VAM infecting these crops.

Levels of VAM infection of wheat varied at the different study sites. Although, these differences might be related to environmental conditions such as soil moisture or nutrient levels, there were no consistent trends of VAM infection with respect to the soil moisture and temperature gradient of the four soil zones. Furthermore, there was no correlation between VAM infection of wheat and soil properties such as organic matter, NO3-N and plant available P. Hayman et al. (1976) also failed to observe any correlation between VAM infection and soil fertility over a range of field sites, and speculated that natural variability of VAM propagule distribution could explain site to site variation in VAM infection. My results support this suggestion as infected wheat roots at different sites contained vesicles of different shapes, indicating infection by different species, and a number of VAM species were detected at different field sites.

Seven VAM species were isolated from six soils after enrichment of indigenous VAM by propagation on maize. Based on the morphological characteristics of spores these were classified into species (described in literature) with which our isolates showed closest resemblances (Schenck and Perez 1988). Glomus spp. were the most common VAM in Saskatchewan soils. Glomus species are reported to be the most common VAM
throughout the world (Gerdemann and Trappe 1974; Blaszkowski 1989) and readily detected in association with monocotyledonous plants (Schenck and Kinloch 1980). Thus the frequent detection of *Glomus* spp. in the wheat field soil was not surprising.

The number of VAM species I isolated from Saskatchewan agricultural soils was relatively small compared to a study by Ellis et al. (1992) who reported the occurrence of 26 VAM species in field soils from a grain sorghum-soybean rotation in Nebraska. However, similar numbers of VAM species (i.e., 3-6) were observed in agricultural field soils in Australia (Abbott and Robson 1977a), corn field soils in Mexico (Berch et al. 1989) and sagebrush-grasslands in Wyoming (Stahl and Christensen 1982). One weakness of my study on VAM distribution was that indigenous VAM spore mixtures were enriched in the rhizosphere of maize. This demonstrates the occurrence of these VAM in those soils. However, it is possible that (i) other VAM exist in Saskatchewan soils but did not grow in association with maize, (ii) the VAM detected were not the most abundant but rather stimulated by maize, and (iii) these VAM may not impact on the growth of wheat or lentil. For example, I rarely detected *G. decipiens* spores in direct extract of field soils, but these spores were detected in five soils after enrichment on maize.

It is difficult to ascertain the positive role of VAM in wheat and lentil grown in Saskatchewan from the results of my survey. Nevertheless, this study provides interesting observations on the status of VAM in Saskatchewan field soils. Vesicular-arbuscular mycorrhizal fungi were widespread in wheat and lentil grown in Saskatchewan and their activity varied from site to site and crop to crop. Studies on the ability of these VAM species to infect wheat and lentil alone or in combination under controlled and field conditions will help demonstrate their impact on crop growth.
3.2 Effects of crop rotation and soil phosphorus levels on vesicular-arbuscular mycorrhizae in field grown crops in Saskatchewan

3.2.1 Introduction

Vesicular-arbuscular mycorrhizae (VAM) improve the phosphorus nutrition of plants grown in soils low in available P (Harley and Smith 1983). Although VAM naturally occur in agricultural soils (Mosse et al. 1981), different agricultural practices such as cultivation of a non-host crop (e.g., canola) in rotation with a host crop (e.g., wheat or barley), summerfallow or agrichemical applications may decrease spore numbers or propagule density of VAM (Tester et al. 1987; Abbott and Robson 1991a). Inoculation of crops with VAM may increase yields under field conditions (Fitter 1985; McGonigle 1988). Most experiments in which crop growth increased from VAM inoculation were conducted in fields where natural inoculum levels were low, following either soil sterilization (Hattingh and Gerdemann 1975; La Rue et al. 1975), fallowing or previous growth of non-host crops (Khan 1975; Gerdemann 1975; Black and Tinker 1979; Clarke and Mosse 1981).

Wheat and barley are two important crops grown in a rotation with canola or after summerfallow in Saskatchewan. Vesicular-arbuscular mycorrhizae are widespread in the rhizosphere of wheat grown in Saskatchewan agricultural soils (see section 3.1). Kucey (1981) surveyed VAM in Saskatchewan and found that canola or summerfallow reduced spore numbers in agricultural soils. However, his study was limited and little is known about the effects of crop rotation and soil fertility on VAM population dynamics in Saskatchewan agroecosystems. My study evaluated the effects of canola or summerfallow on VAM spore numbers and subsequent infection of wheat and barley in Saskatchewan fields. The relationship between available soil P and VAM infection of wheat and lentil grown on fields along a catenary sequence also was evaluated.
3.2.2 Materials and Methods

3.2.2.1 Effect of canola or summerfallow on VAM infection of wheat and barley crops

Vesicular-arbuscular mycorrhizal infection in wheat (*Triticum aestivum* L. cv. Katepwa) and barley (*Hordeum vulgare* L cv. Argyle) grown in field soils previously cropped to wheat or canola, or kept in summerfallow were monitored during the 1988 and 1989 field season. Wheat was studied at three sites during 1988, and wheat and barley were each studied at three sites during 1989 (Table 3.2.2.1). The study sites represented Dark Brown, Black and Grey Chernozemic soils. In each study site soil and root samples were collected from the adjacent wheat fields which were cropped to wheat, or canola or kept summerfallow in the previous year. Wheat and barley plants were taken from a 100 m\(^2\) area (Fig. 3.2.2.1).

Plants were sampled three times during the growing season at times coinciding with various physiological crop stages (tillering, panicle emergence and maturity). The crop was sampled four times at the Englefeld site during 1988; the additional sample was taken in between tillering and panicle emergence. Twenty plants were collected randomly from a 100 m\(^2\) area at each wheat and barley field for each study site. The roots and surrounding soil were excavated to a depth of 15-20 cm. The roots were washed free of soil and 20 samples from each site combined and stored at -30 °C for 3 months before an estimation of VAM infection was made. Soil samples (10 cores of 2.5 cm diam × 15-20 cm length) also were collected (at the time of first sampling interval) from the 100 m\(^2\) area of each field, bulked together, air dried and the composite samples used to determine soil characteristics for each site (Table 3.2.2.1). Root sampled were processed and VAM infection estimated as described in section 3.1.2.2.
Table 3.2.2.1 Soil characteristics of wheat and barley field sites under different rotations

<table>
<thead>
<tr>
<th>Site</th>
<th>Crop rotation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Soil&lt;sup&gt;b&lt;/sup&gt;</th>
<th>pH</th>
<th>NO&lt;sub&gt;3&lt;/sub&gt;-N</th>
<th>Available P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1987</td>
<td>1988</td>
<td>1989</td>
<td></td>
<td>μg g⁻¹</td>
</tr>
<tr>
<td>Aberdeen</td>
<td>W</td>
<td>W</td>
<td>NS</td>
<td>7.4</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>SF</td>
<td>W</td>
<td>NS</td>
<td>7.4</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>W</td>
<td>NS</td>
<td>7.2</td>
<td>59</td>
</tr>
<tr>
<td>Englefeld</td>
<td>W</td>
<td>W</td>
<td>NS</td>
<td>7.5</td>
<td>106</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>W</td>
<td>NS</td>
<td>7.5</td>
<td>117</td>
</tr>
<tr>
<td>Glenavon</td>
<td>W</td>
<td>W</td>
<td>NS</td>
<td>7.2</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>CN</td>
<td>W</td>
<td>NS</td>
<td>7.2</td>
<td>54</td>
</tr>
<tr>
<td>Lucky Lake</td>
<td>NS</td>
<td>W</td>
<td>W</td>
<td>7.1</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>SF</td>
<td>W</td>
<td>7.2</td>
<td>37</td>
</tr>
<tr>
<td>Watrous</td>
<td>NS</td>
<td>W</td>
<td>W</td>
<td>7.4</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>SF</td>
<td>W</td>
<td>7.4</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>CN</td>
<td>W</td>
<td>7.3</td>
<td>42</td>
</tr>
<tr>
<td>Stenen No. 1</td>
<td>NS</td>
<td>W</td>
<td>W</td>
<td>7.3</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>CN</td>
<td>W</td>
<td>7.2</td>
<td>60</td>
</tr>
<tr>
<td>Semans</td>
<td>NS</td>
<td>W</td>
<td>B</td>
<td>7.1</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>CN</td>
<td>B</td>
<td>7.1</td>
<td>51</td>
</tr>
<tr>
<td>Stenen No. 2</td>
<td>NS</td>
<td>W</td>
<td>B</td>
<td>7.4</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>CN</td>
<td>B</td>
<td>7.4</td>
<td>69</td>
</tr>
<tr>
<td>Medstead</td>
<td>NS</td>
<td>W</td>
<td>B</td>
<td>7.5</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>SF</td>
<td>B</td>
<td>7.5</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>CN</td>
<td>B</td>
<td>7.5</td>
<td>75</td>
</tr>
</tbody>
</table>

<sup>a</sup>Wheat was sampled at the first three sites in 1988 and wheat and barley were sampled at the remaining sites in 1989.

<sup>b</sup>Soil samples were taken at the time of tillering.

Note: W = wheat, CN = canola, SF = summerfallow, NS = not studied.
In addition to VAM infection, VAM spore numbers in soils at the time of seeding and harvest of the 1989 wheat and barley crops also were determined. Thirty cores (38 samples, four replicate 100-g sub samples were used for extraction of spores. Mixtures of sporocarps, spores, soil and organic matter were obtained by wet sieving and decanting (53-300 μm) of soil suspensions (1000 mL) (Gerdemann and Nicolson 1963). Spores and sporocarps were cleared of organic detritus and soil particles by sucrose density gradient centrifugation (Daniels and Skipper 1982) and washing, and suspended in tap water. These suspensions were sonicated for 30 sec (at 100 watts) in an ultrasonifier (Braun Sonic 1510) to separate the spores from the sporocarps. Twenty mL of spore suspension were distributed on two 0.8-μm gridded millipore filters (Cellulose nitrate membrane filter, diam. 45-mm, Cat. no. AAWG 047 00, Millipore Copn., Bedford, MA 01730) and spores were counted with a stereomicroscope (47×). Spore numbers are reported on the basis of oven dry (24 h at 80°C) weight of soil.

3.2.2.2 Relationship between available P and VAM infection

A 500 m² area (100 m length × 5 m width) (Fig. 3.2.2.2) was selected along the shoulder, mid and bottom slope position of the wheat fields at Lucky Lake, Watrous, Englefeld and Stenen, and of the lentil fields at Semans, Englefeld and Glenavon. At each slope position three 1 m² areas were randomly selected.

During the growing season, five random wheat plants were collected at tillering, panicle emergence, and harvest from the 1 m² area. Lentil was sampled at flowering, pod formation and seed maturity. At the time of 1st sampling, soil samples (5 cores of 2.5 cm diam × 15-20 cm length) were collected from each 1 m² area. Samples were bulked, broken and uniformly mixed and stored at 7°C until analysed for available P. The root samples from each plant were washed free of soil, bulked and stored at -30°C for 5 months before an estimation.
Figure 3.2.2.1 A typical field with three adjacent crop rotations (Aberdeen site).
Figure 3.2.2.2 Typical catenary sequence sampled at wheat and lentil field sites.
of VAM infection was made. Roots were stained and the percent infection estimated by the grid-line intersect method (Giovannetti and Mosse 1980). The plant available soil P at seeding and percentage of the infected root length of samples collected at each 1 m² area were correlated.

3.2.3 Results

3.2.3.1 Effect of canola or summerfallow on VAM infection of wheat and barley crops

The number of VAM spores in soils under different crop rotations is presented in Table 3.2.3.1. No attempt was made to identify the spores. Spore numbers at seeding of the 1989 wheat and barley fields were lower at all the study sites if the previous crop was canola or the field was kept summerfallow. Fewer spores were recovered from canola field soils than summerfallow fields at the sites where all three rotations were present (adjacent fields). At harvest of the 1989 wheat and barley crops, VAM spore number had increased from the values determined at seeding (Table 3.2.3.2). However, the number of spores in the wheat and barley field soil was lower if previously cropped to canola compared to wheat.

The percentage VAM infection in the 1988 and 1989 wheat crop was reduced when the previous year was either kept summerfallow or cropped to canola (Fig. 3.2.3.1). A similar trend in VAM infection was noted for the 1989 barley crop (Fig. 3.2.3.1). The reduction in VAM infection was more conspicuous at the second and third sampling at most sites. Canola caused a greater reduction in percent VAM infection in both wheat and barley than summerfallow (Fig. 3.2.3.1). The observations were consistent for all sites in different soil zones.
Table 3.2.3.1 Number of VAM spores in canola, summerfallow and wheat field soils

<table>
<thead>
<tr>
<th>Site</th>
<th>Canola</th>
<th>Summerfallow</th>
<th>Wheat</th>
<th>Mean difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>____</td>
<td>____</td>
<td>____</td>
<td>____</td>
</tr>
<tr>
<td>Lucky Lake</td>
<td>NS</td>
<td>535 ± 57</td>
<td>787 ± 61</td>
<td>252</td>
</tr>
<tr>
<td>Watrous</td>
<td>950 ± 39</td>
<td>1085 ± 57</td>
<td>NS</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>950 ± 39</td>
<td>NS</td>
<td>1227 ± 44</td>
<td>277</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>1085 ± 57</td>
<td>1227 ± 44</td>
<td>142</td>
</tr>
<tr>
<td>Stenen No. 1</td>
<td>1515 ± 113</td>
<td>NS</td>
<td>1852 ± 70</td>
<td>337</td>
</tr>
<tr>
<td>Semans</td>
<td>957 ± 43</td>
<td>NS</td>
<td>1220 ± 44</td>
<td>263</td>
</tr>
<tr>
<td>Medstead</td>
<td>410 ± 53</td>
<td>542 ± 43</td>
<td>NS</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>410 ± 53</td>
<td>NS</td>
<td>892 ± 101</td>
<td>482</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>542 ± 43</td>
<td>892 ± 101</td>
<td>350</td>
</tr>
<tr>
<td>Stenen No. 2</td>
<td>1580 ± 112</td>
<td>NS</td>
<td>1837 ± 70</td>
<td>257</td>
</tr>
</tbody>
</table>

*a Soil samples were collected at the time of seeding of 1989 wheat crop.

*b Oven dry basis.

Note: NS = not studied; ± = standard deviation of four sub-samples.
Table 3.2.3.2 Number of VAM spores in wheat and barley field soils previously cropped to canola or wheat, or kept in summerfallow

<table>
<thead>
<tr>
<th>Site</th>
<th>1988 crop</th>
<th>1989 crop</th>
<th>No. of spores at seeding</th>
<th>No. of spores at harvest</th>
<th>Mean increase in spore no. per 100 g soil&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lucky lake</td>
<td>Wheat</td>
<td>Wheat</td>
<td>787 ± 61</td>
<td>1105 ± 76</td>
<td>318</td>
</tr>
<tr>
<td></td>
<td>Summerfallow</td>
<td>Wheat</td>
<td>535 ± 57</td>
<td>805 ± 69</td>
<td>270</td>
</tr>
<tr>
<td>Watrous</td>
<td>Wheat</td>
<td>Wheat</td>
<td>1227 ± 44</td>
<td>1468 ± 61</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>Summerfallow</td>
<td>Wheat</td>
<td>1085 ± 57</td>
<td>1193 ± 42</td>
<td>108</td>
</tr>
<tr>
<td></td>
<td>Canola</td>
<td>Wheat</td>
<td>950 ± 39</td>
<td>1108 ± 66</td>
<td>158</td>
</tr>
<tr>
<td>Stenen No. 1</td>
<td>Wheat</td>
<td>Wheat</td>
<td>1852 ± 70</td>
<td>2113 ± 136</td>
<td>261</td>
</tr>
<tr>
<td></td>
<td>Canola</td>
<td>Wheat</td>
<td>1515 ± 113</td>
<td>1853 ± 131</td>
<td>338</td>
</tr>
<tr>
<td>Semans</td>
<td>Wheat</td>
<td>Barley</td>
<td>1220 ± 44</td>
<td>1638 ± 64</td>
<td>418</td>
</tr>
<tr>
<td></td>
<td>Canola</td>
<td>Barley</td>
<td>957 ± 43</td>
<td>1213 ± 55</td>
<td>256</td>
</tr>
<tr>
<td>Medstead</td>
<td>Wheat</td>
<td>Barley</td>
<td>892 ± 101</td>
<td>1090 ± 147</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>Summerfallow</td>
<td>Barley</td>
<td>542 ± 43</td>
<td>800 ± 64</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>Canola</td>
<td>Barley</td>
<td>410 ± 53</td>
<td>623 ± 146</td>
<td>213</td>
</tr>
<tr>
<td>Stenen No. 2</td>
<td>Wheat</td>
<td>Barley</td>
<td>1837 ± 70</td>
<td>2145 ± 172</td>
<td>308</td>
</tr>
<tr>
<td></td>
<td>Canola</td>
<td>Barley</td>
<td>1580 ± 112</td>
<td>1873 ± 123</td>
<td>293</td>
</tr>
</tbody>
</table>

<sup>a</sup> Oven dry basis.
Note: ± = standard deviation of four sub-samples.
Figure 3.2.3.1 Effect of previous year crop on VAM infection in wheat and barley grown in the subsequent year; wheat (●), canola (○) or summerfallow (□) in the previous year. Infection values are means of three subsamples ± S.D.
3.2.3.2 Relationship between available P and VAM infection

The relationship between the soil available P status and the percent VAM infection of lentil and wheat at three sampling times at different study sites is shown in Fig. 3.2.3.2. The statistically significant negative correlation indicates that VAM infection of plant roots decreased as the available P level increased.

3.2.4 Discussion

Agricultural practices in Saskatchewan, such as production of canola or summerfallow in rotation with wheat and barley influenced the population dynamics of indigenous VAM (i.e., spores numbers) resulting in reduced infection of subsequent wheat and barley. There was an 11-39% reduction in VAM spore number in summerfallow field soils compared to adjacent wheat field soils. Similarly production of canola caused a reduction in spore numbers by 18-54%. Because this study was conducted on adjacent fields with similar soil properties (Table 3.2.2.1), the reduction in VAM spore number was attributed to the absence of a host crop in the previous season. Alternatively, toxic substances from the previous season’s canola residues may be associated with the reduction in spore numbers.

The VAM infection pattern in wheat and barley grown on soils previously cropped to canola or kept in summerfallow followed the same three phase pattern previously observed for wheat (Section 3.1.3.2), but the level of infection was lower than in a continuous wheat rotation. Black and Tinker (1979) studied the effect of non-host crops and summerfallow on VAM, and found twice as many spores at the end of the season in soils of barley plots compared to summerfallow and non-mycorrhizal kale (Brassica oleracea L.) plots. Similarly, Harinikumar and Bagyaraj (1988) found that leaving the land fallow for one season reduced mycorrhizal propagules by 40% while growing non-
Figure 3.2.3.2. Relationship between available P and % VAM infected root length for wheat and lentil at 20 (A), 50 (B) and 70 (C) days after emergence.
mycorrhizal mustard [Brassica juncea (L) Czern. and Coss.] caused a 13% reduction. Baltruschat and Dehne (1988) also observed that inclusion of a non host crop in a continuous wheat rotation reduced the VAM inoculum potential of the soil. In contrast, Hayman et al. (1975) found no drastic reduction in spore numbers after cultivation of a non host crop, and suggested that many VAM spores remain viable for at least one year. In all of these studies, the reasons for a reduction in spore numbers following fallow or non-host crops are not known. Destruction of spores by saprophytic soil microorganisms is one possible explanation (Ross and Ruttencutter 1977).

Cultivation of wheat or barley increased VAM spore numbers in soil by 10-50%, and 16-52%, respectively. The largest increase occurred in fields previously cropped to canola or kept summerfallow. Thus long term impact of non mycorrhizal crops or summerfallow is probably negligible, and can be mitigated by growing a suitable host crop. I made no attempt to evaluate the effect of the crop rotations on population dynamics of specific VAM species, but such studies might prove interesting.

My results indicated that VAM infection levels in wheat and barley were associated with initial spore numbers. Black and Tinker (1979) also observed a relationship between VAM spores in soil and the rate of spread and the level of VAM infection in barley grown in fields previously cropped to kale or kept summerfallow. They suggested that reduced VAM spore numbers at seeding of barley not only decreased total VAM infection but also reduced the rate of VAM infection.

The numbers of VAM spores recovered from soils in this study were much higher than those obtained in my previous study (see Section 3.1). However, in this study VAM spores were released from sporocarps by sonication of spore suspensions, whereas in the previous study sporocarps were counted as single spore. Whole sporocarps, or the fragments of sporocarps of G. fasciculatum and G. versiforme contain on average 30 to
179 spores (unpublished data). Furthermore, in this study I also counted the spores floating on top of the interface between two sucrose gradients (20% and 60%). Approximately 37% of *G. mosseae* and 8% of *G. versiforme* spores float on the top of the interface during the density gradient centrifugation (unpublished data). The floated spores of *G. mosseae* were viable as these spores produced infection in maize in sterile soil (unpublished data). This may be the reason why the number of spores recovered from soil in this study was much higher than in the previous study.

High levels of P fertilizer reduced VAM infection of crops in field experiments (Jensen and Jacobsen 1980; Abbott and Robson 1991a). Yet most survey studies found little correlation between the available soil P level and VAM infection of field grown crops (Hayman 1982a; Abbott and Robson 1991a). I also failed to find any correlation between available soil P and VAM infection of the wheat at different field sites in my previous survey study (see section 3.1). However, using detailed sampling techniques I obtained a correlation between available P levels in the wheat and lentil field soils and the level of VAM infection in these crops. This result suggests that soil available P in the vicinity of roots may influence VAM infection in roots, probably roots in a high available P zones do not depend to great extent on VAM for P absorption.

This study shows that the VAM spore populations in soils and crops grown at agricultural fields under similar environmental and soil conditions fluctuate from one season to another depending upon the cultivation of canola or the summerfallow break in continuous wheat and barley rotation. Available P along the catenary sequence was negatively correlated with infection in barley and lentil roots.
3.3 Propagation and storage of propagules of vesicular-arbuscular mycorrhizae isolated from Saskatchewan agricultural soils

3.3.1 Introduction

Vesicular-arbuscular mycorrhizae (VAM) are a group of obligate plant symbionts that influence plant growth and development. Vesicular-arbuscular mycorrhizal species are known to differ in their ability to increase plant growth (Mosse 1972; Abbott and Robson 1978; Powell 1982; Sylvia and Burks 1988). These differences may be related to environmental conditions and cropping history, or the inherent characteristics of a specific VAM isolate. Therefore, the isolation and identification of efficient VAM strains from a particular region may be an important aspect in application of VAM to crop production. Selection and evaluation of efficient VAM strains from specific soils require the development of suitable methods for production and storage of monospecific cultures of individual VAM strains.

The production of VAM inocula is influenced by many factors such as host species (Bagyaraj and Manjunath 1980a; Hetrick and Bloom 1986; Struble and Skipper 1988), growth medium and nutrient levels (Menge et al. 1978a; Ferguson 1981; Douds and Schenck 1990), light intensity (Ferguson and Menge 1982) and temperature (Furlan and Fortin 1973). Furthermore, the procedures used to extract spores from a soil-root mixture and the conditions under which VAM inocula are stored may affect inoculum viability (Ferguson and Woodhead 1982; Daft et al. 1987). For example, sucrose density gradient centrifugation is often used to remove debris from spore mixtures prior to assessing the efficiency of different VAM species. The effect of sucrose density gradient centrifugation on the viability and infection potential of VAM spores is not well documented although long exposure of VAM spores to 60% (v/v) glycerol solution
causes plasmolysis (Furlan et al. 1980). I have isolated seven VAM strains from agricultural soils of Saskatchewan (Section 3.1). To assess the efficacy of these VAM isolates to promote growth of wheat and lentil grown in Saskatchewan required production of monospecific cultures of these VAM. Here I report factors necessary to optimize the production of three monospecific VAM inoculants. The effects of storage conditions and spore extraction procedures on the viability of spores were also assessed.

3.3.2 Materials and Methods

3.3.2.1 Optimization of spore production by *Glomus geosporum*

A growth chamber experiment assessed the influence of factors such as host crop, soil volume, and soil sterilization on production of *G. geosporum* Nicolson and Gerdemann spores. The host crops were grown in a soil:sand mix which was prepared by mixing an Outlook soil (Bradwell association, sandy texture, 10 μg available P g⁻¹) with a coarse sand (Silica sand grade #7 Target Product Ltd., Calgary - AB, Canada) in equal proportion. The soil:sand mix was amended with 50 mL of a modified Hoagland solution kg⁻¹ mix (section 3.1.2.3) to compensate for original soil nutrient levels, and sterilized (twice for 1 h at 121°C at an interval of 24 h) to eliminate indigenous VAM. Three host crops i.e., lentil (*Lens esculenta* L. cv. 'Eston'), maize (*Zea mays* L. cv. 'Early golden bantam') and a sorghum [*Sorghum bicolor* (L.) Moench] - sudangrass [*Sorghum sudanense* (Piper) Hitch.] hybrid cv. 'Graze all' were assessed. The effect of soil volume was tested by growing maize in 2 kg (pot size, 15 cm top diam × 15 cm height) and 6 kg (pot size, 22 cm top diam × 21 cm height) of soil:sand mix. Two methods of sterilization were evaluated (steam sterilization for 1 h and autoclaving as described earlier). The properties of the soil:sand mix before and after sterilization and are presented in Table 3.3.2.1. Autoclaving increased available Mn content than steaming
Table 3.3.2.1. Characteristics of soil:sand (1:1) mix before and after sterilization\(^a\)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>Cond.</th>
<th>NO(_3)-N</th>
<th>P(^b)</th>
<th>K</th>
<th>SO(_4)-S</th>
<th>Cu</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before sterilization</td>
<td>7.8</td>
<td>0.3</td>
<td>37.4</td>
<td>10.5</td>
<td>285</td>
<td>9.1</td>
<td>0.42</td>
<td>7.68</td>
<td>0.70</td>
<td>8.66</td>
</tr>
<tr>
<td>Autoclave sterilization</td>
<td>7.5</td>
<td>0.4</td>
<td>34.3</td>
<td>11.2</td>
<td>335</td>
<td>10.4</td>
<td>0.32</td>
<td>4.46</td>
<td>0.64</td>
<td>71.40</td>
</tr>
<tr>
<td>Steam sterilization</td>
<td>7.6</td>
<td>0.4</td>
<td>35.8</td>
<td>11.0</td>
<td>321</td>
<td>10.5</td>
<td>0.40</td>
<td>5.89</td>
<td>0.64</td>
<td>39.40</td>
</tr>
</tbody>
</table>

\(^a\) Determined by Saskatchewan Soil Testing Laboratory. Values are means of three subsamples.

\(^b\) P, plant available.
perhaps due to breakdown of Mn-minerals present in the soil. Various combinations of
the above factors resulted in six treatments (Table 3.3.3.1) which were replicated four
times in a completely randomized design. The treatment combinations were chosen based
on preliminary spore production studies.

3.3.2.1.1 Inoculation of host crops and plant growth conditions

Crop seeds were surface sterilized by treating with 70% (v/v) ethyl alcohol for 1
min, 1.2% (w/v) sodium hypochlorite for 5 min and washed 8 times with sterile distilled
water. Seeds were aseptically germinated on 0.3% Trypticase soy agar (0.3% Trypticase
soy broth, 1.5% agar). Two uniform 4-day old seedlings of the host crops were
inoculated with 150 G. geosporum spores using the funnel technique (Menge and Timmer
1982). The G. geosporum strain was isolated from a wheat field soil at Outlook,
Saskatchewan. Spores were propagated on maize (see section 3.2) and stored at 7°C for
two weeks prior to extraction of G. geosporum spores. Plants were grown with a 14 h
day-cycle for the first week, a 15 h day for the second and third week, and a 16 h day
thereafter. The day and night temperatures were 23°C and 18°C for the first 3 weeks and
25°C and 20°C thereafter. The light intensity in the growth chamber during the growing
period ranged from 470-480 μE m⁻² s⁻¹. Pots were supplied with 50 mL of a modified
Hoagland solution (see section 3.1) kg⁻¹ soil:sand mix every week up to the eight weeks.
Lentil plants also received 50 mL of this solution on the 9th and 10th weeks. The
soil:sand mix was maintained at 70% moisture holding capacity (MHC) (ca. - 60 kPa soil
water potential) and watered daily with sterile distilled water to constant weight.

3.3.2.1.2 Growth parameters, VAM infection and spore production

Maize and sorghum-sudangrass hybrid plants were harvested at 90 days and lentil
plants at 98 days after planting (DAP). Shoots were oven dried (72 h at 54°C) and
weighed. Senesced leaves of lentil also were collected from each pot, oven dried,
weighed and included in the total shoot dry wt of lentil. The soil-sand-root mixtures from each pot were air dried at 24°C for one week, broken, free roots cut into small pieces and thoroughly mixed. Two 200-g sub samples of this "inoculum mixture" were sieved (500 μm), roots recovered, dried (72 h at 54°C) and dry weight of roots for the entire pots estimated.

The VAM infection of crop roots and spore numbers for each treatment were determined on two 50-g air dry samples of the inoculum mixture. Roots were cleared in 5% (w/v) KOH, stained in a lactoglycerol trypan blue solution (Phillips and Hayman 1970) and percent infection estimated by the grid line intersect method (Giovannetti and Mosse 1980). The dry weight of infected roots per pot was estimated (dry wt of roots x estimated percentage of the roots colonized). Spores were extracted by wet sieving and sucrose density gradient (20% and 60%) centrifugation (Daniels and Skipper 1982) and suspended in water. The spore suspension was made up to 100 mL. Two 5-mL aliquots were removed from the continuously stirred spore suspensions, and spores in each aliquot recovered on a gridded 0.8 μm millipore filter (Cellulose nitrate membrane filter, diam. 45-mm, Cat. no. AAWG 047 00, Millipore Corp., Bedford, MA 01730). The spores were counted using a stereomicroscope (47×).

3.3.2.1.3 Nutrient content

Shoots (leaves plus stems) were digested in H2SO4-H2O2 (Thomas et al. 1967). Total-N and -P in the digests were determined using an Autoanalyzer II Technicon system (Technicon Industrial Systems, Tarrytown, N.Y., U.S.A.).

3.3.2.2 Production of monospecific cultures of G. geosporum, G. mosseae and G. versiforme

*Glomus geosporum, G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe and *G. versiforme* (Karsten) Berch were propagated using maize as the host crop grown in
plastic pots containing 2 kg of autoclaved soil:sand mix (Table 3.3.2.1). *Glomus geosporum* (above strain) and *G. mosseae* spores were isolated from a wheat field soil at Outlook and *G. versiforme* spores from a wheat field at Semans, Saskatchewan (Section 3.2). The inocula were stored at 7°C for 110 days before extraction of spores. Maize seeds were surface sterilized, germinated and the seedlings inoculated and grown as described above. Plant growth conditions, harvest and spore recovery were as above, except that the light intensity ranged from 290-350 µE m⁻²s⁻¹ during crop growth. To prevent contamination of monospecific cultures the pots were maintained inside large plastic containers as shown in Fig 3.3.2.1.

At harvest, spores of the three VAM strains were recovered (as above) from three 25-g sub samples of the monospecific cultures and the numbers of spores in various size classes determined.

### 3.3.2.3 Effects of temperature and osmotic stress on *G. mosseae* spores

The effects of storage and osmotic stress on the viability of *G. mosseae* spores were evaluated by using wheat and sorghum - sudangrass hybrid as test crops. A monospecific culture of *G. mosseae* was air dried at 25°C for 30 days, the roots cut into small pieces and the inoculum thoroughly mixed. This inoculum was divided in two halves. One half was stored at 7°C and the other half was stored at 25°C for 120 days.

Mycorrhizal spores were extracted by wet sieving and decanting (Gerdemann and Nicolson 1963) and centrifuged for 5 min at 2500 rpm (approx 350 × g). The spore-debris mix of the inoculum stored at 7°C was divided into two parts. One part was used to collect spores (without osmotic stress). The other part was cleared of debris by 20% and 60% sucrose density gradient centrifugation as described above. During this step spores were exposed to each sucrose concentration for 6-8 min (Daniels and Skippers 1982). Thus, each crop was inoculated with three types of treated spores. These
Figure 3.3.2.1 Pot system for production of monospecific VAM cultures.
included spores stored at 7°C without osmotic stress, at 7°C subjected to osmotic stress and at 25°C without osmotic stress. Spores were collected with the help of a stereomicroscope (47x). The four treatments (including an uninoculated control) for each crop were replicated four times in a completely randomized design.

3.3.2.3.1 Inoculation and growth conditions of plants

Seeds of wheat and the sorghum-sudangrass hybrid were surface sterilized and germinated as above. Two uniform four-day old seedlings of each crop were inoculated (as above) with 150 G. mosseae spores. The control plants of the two crops received 5 mL of a water filtrate obtained by passing a suspension of spores and organic debris (obtained after wet sieving and decanting of monospecific cultures stored at 7°C) through a 1.2 μm millipore filter (cellulose nitrate membrane filter, diam. 25-mm, Cat no. RAWP 025 00, Millipore Corp., Bedford, MA 01730). The plants were grown in styrofoam cups containing 450 g autoclaved (1h at 121°C) soil:sand mix (Table 3.3.2.1). Plants were grown with a 16 h day (light intensity of 350-390 μE m⁻² s⁻¹; 24°C day and 18°C night). Plants were fertilized weekly with 25 mL of one-half strength of a modified Hoagland solution (Section 3.2) for the first four weeks. The growth medium was maintained at 70% MHC and watered daily with sterile distilled water to constant weight.

3.3.2.3.2 Estimation of VAM infection and plant parameters

Plants were harvested at day 87, dried at 54°C for 72 h and shoot, grain and root weights determined. Sorghum plants did not produce grain by the 87th day. Percent VAM infection was determined on 0.02 g dry root samples, and spores were extracted from the soil:sand mix and counted as above.

3.3.2.4 Statistical analysis

Data were analysed by separate one way analysis of variance. When F values were
significant, the least significant difference test was used to separate the means (Little and Hills 1979)

3.3.3 Results

3.3.3.1 Optimization of spore production by *Glomus geosporum*

Different numbers of *G. geosporum* spores were produced in the soil:sand used to grow lentil, maize, and the sorghum-sudangrass hybrid. The maize roots in uninoculated control pots had no VAM infection. This indicated that sterilization of soil:sand was effective in elimination of indigenous VAM from the growth medium. Under similar growth conditions, maize supported production of twice as many VAM spores (per g of soil:sand mix) as the sorghum-sudangrass hybrid and four times as many as lentil (Table 3.3.3.1). The amount of VAM infected roots for these crops followed a similar trend: maize > sorghum-sudangrass hybrid > lentil.

Soil volume and nutrient availability influenced VAM spore production on the maize plants. Significantly more spores were produced per g soil:sand in 2 kg (soil:sand mix) pots than in 6 kg (soil:sand mix) pots (Table 3.3.3.1). In fact, there was only a 25% increase in spore production when the soil volume was increased 3-fold (i.e., from 2 to 6 kg). Spore production was related to plant growth and nutrient availability. Maize grew better in the 6 kg pots and yielded more shoot material (Table 3.3.3.1) and produced cobs with kernels (data not shown). However, these plants also had a lower root:shoot ratio (Table 3.3.3.1). Maize plants grown in the 2 kg pots exhibited symptoms of nutrient deficiency (i.e., P and N, Fig. 3.3.3.1) early in the growth cycle, and the severity of this deficiency increased as plants matured. These plants did not produce cobs with kernels. Shoot materials of plants grown in 6 kg and 2 kg soil:sand mix had similar N and P levels
Table 3.3.3.1 Production of *Glomus geosporum* spores as influenced by host crop and soil volume

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Host crop</th>
<th>Soil volume (kg)</th>
<th>Sterilization method</th>
<th>Plant dry weight (g)</th>
<th>Nutrient content (mg g&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Nutrient uptake (mg per pot)</th>
<th>No. of spores&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Total spore (x10&lt;sup&gt;3&lt;/sup&gt; per pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Infected root</td>
<td>Root:shoot ratio (%)</td>
<td>Infection (%)</td>
</tr>
<tr>
<td>Maize 2</td>
<td>Maize</td>
<td>Autoclaving&lt;sup&gt;d&lt;/sup&gt;</td>
<td>45.72</td>
<td>14.36</td>
<td>0</td>
<td>0.31</td>
<td>0</td>
<td>4.80</td>
</tr>
<tr>
<td>Maize 6</td>
<td>Maize</td>
<td>Autoclaving</td>
<td>78.08</td>
<td>22.26</td>
<td>7.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28</td>
<td>33.8</td>
<td>5.06</td>
</tr>
<tr>
<td>Maize 2</td>
<td>Maize</td>
<td>Autoclaving</td>
<td>46.27</td>
<td>18.02</td>
<td>9.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.39</td>
<td>53.2</td>
<td>5.10</td>
</tr>
<tr>
<td>Maize 2</td>
<td>Maize</td>
<td>Steaming&lt;sup&gt;e&lt;/sup&gt;</td>
<td>45.77</td>
<td>17.42</td>
<td>9.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38</td>
<td>57.5</td>
<td>4.99</td>
</tr>
<tr>
<td>Sorghum 2</td>
<td>Maize</td>
<td>Autoclaving</td>
<td>21.12</td>
<td>8.81</td>
<td>3.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.42</td>
<td>34.4</td>
<td>-</td>
</tr>
<tr>
<td>Lentil 2</td>
<td>Maize</td>
<td>Autoclaving</td>
<td>10.07</td>
<td>3.98</td>
<td>2.80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.46</td>
<td>69.1</td>
<td>-</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.40</td>
<td>10.3</td>
<td>ns</td>
</tr>
</tbody>
</table>

<sup>a</sup> All treatments received 150 *G. geosporum* spores except Treatment No. 1 which was an uninoculated control.

<sup>b</sup> Spores were extracted from two 50-g sub samples of monospecific cultures (soil, root and spore mixture) of each isolate and made to 100 mL in tap water. Numbers estimated by counting spores in two 5-mL aliquots.

<sup>c</sup> Estimated as dry wt of roots x percentage of root colonized.

<sup>d</sup> Autoclaved twice for 1 h at 121°C at an interval of 24 h.

<sup>e</sup> Steamed for 1 h.

<sup>f</sup> Values are means of four replications; numbers within a column followed by the same letter are not significantly different (P = 0.05).
Figure 3.3.3.1 Nutrient deficiency symptoms of 56 day old maize plants grown in 2 kg soil:sand mix. Note yellow purple color of leaves.
at harvest (Table 3.3.3.1). However, uptake of these nutrients relative to the available levels in the total soil volume differed significantly, and resulted in severe stress for plants grown in 2 kg pots. There was 22.4 and 67.2 mg of available P in the 2 kg pots and 6 kg pots, respectively (Table 3.3.2.1), but shoot growth required 37.3 and 69.3 mg P (Table 3.3.3.1). Thus plants in 2 kg pots had to "mine" the soil for 40% of their shoot P requirement, whereas plants in 6 kg pots had to extract only 3% of their requirement. Similarly, 45% and 2% of the maize N requirement was obtained from the soil in 2 and 6 kg pots, respectively. These nutrients in excess of the amounts available in the 2 kg pots were probably obtained by the plants from the slowly available pools.

Sterilizing the soil:sand mixture by autoclaving increased the Mn content ca. 9-fold whereas steam treatment caused a 5-fold increase in Mn content. Sterilization procedure had no effect on spore production (Table 3.3.3.1).

3.3.3.2 Production of monospecific cultures of G. geosporum, G. mosseae and G. versiforme

Monospecific cultures were produced using maize grown in 2 kg of autoclaved soil:sand mix. The light intensity of the growth chamber in this experiment (290-350 μE m^{-2} s^{-1}) was lower than that of the earlier experiment (470-480 μE m^{-2} s^{-1}). Glomus geosporum produced more spores on the maize than G. versiforme and G. mosseae (Table 3.3.3.2). However, in this study G. geosporum produced only ca. 67% the number of spores produced in the earlier experiment conducted to optimize G. geosporum spore production (Table 3.3.3.1).

Spores produced by the three VAM species varied in size (Figs. 3.3.3.2 and 3.3.3.3). However, the majority of the spores for any one species fell into a narrow size category. For example, individual spores of G. geosporum ranged in size from 53-250 μm, but 83% of spores were in the 107-190 μm size range. Similarly, 62% of the spores
Table 3.3.3.2 Spore production and infection level of Glomus geosporum, Glomus mosseae and Glomus versiforme grown on maize<sup>a</sup>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot dry wt (g)</th>
<th>Infection (%)</th>
<th>No. of spores&lt;sup&gt;b&lt;/sup&gt; (g⁻¹ soil:sand)</th>
<th>Total spores&lt;sup&gt;b&lt;/sup&gt; (x10³ per pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. geosporum</td>
<td>33.41</td>
<td>54.33&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>179&lt;sup&gt;a&lt;/sup&gt;</td>
<td>358&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G. mosseae</td>
<td>29.15</td>
<td>37.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>G. versiforme</td>
<td>37.24</td>
<td>66.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>180&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>ns</td>
<td>28.5</td>
<td>21</td>
<td>73</td>
</tr>
</tbody>
</table>

<sup>a</sup>Two seedlings were inoculated with 150 spores of each VAM isolate and grown in 2 kg soil:sand (1:1) mix to produce monospecific cultures.

<sup>b</sup>Spores were extracted from two 50-g sub samples of monospecific cultures (soil, root and spore mixture) of each isolate and made to 100 mL in tap water. Numbers estimated by counting spores in two 5-mL aliquots.

<sup>c</sup>Values are means of three replications; numbers within a column followed by the same letter are not significantly different (P = 0.05).
Figure 3.3.3.2 Spores of (A) *Glomus geosporum*, (B) *Glomus mosseae* and (C) *Glomus versiforme* produced in monospecific cultures on a maize host. Bar marker = 300 μm.
Figure 3.3.3.3 Size distribution of (A) *Glomus geosporum*, (B) *Glomus mosseae* and (C) *Glomus versiforme* spores in their monospecific cultures. Spores from a 25-g sample were suspended in tap water and passed through sieves of decreasing pore size. Spores retained on each sieve were counted under a stereomicroscope (47x). Each bar represents the average spore number of three replicate samples.
produced by *G. mosseae* were in the 107-190 μm size, although individual *G. mosseae* spores ranged from 53 to 300 μm. *Glomus versiforme* spores were the smallest, and 86% of these spores were in the size range of 54-106 μm.

### 3.3.3.3 Effect of temperature and osmotic stress on *G. mosseae* spores

*Glomus mosseae* spores stored at 7°C maintained their viability longer than those stored at 25°C. This was reflected by a significantly higher level of infection in wheat and sorghum-sudangrass roots, and also by greater spore production (Table 3.3.3.3). In addition, inoculation with *G. mosseae* spores stored at 7°C increased shoot growth of wheat (Fig. 3.3.3.3) and sorghum-sudangrass whereas spores stored at 25°C had no effect compared to the uninoculated control (Table 3.3.3.3). Osmotic stress (20% and 60% sucrose solution) had no effect on the viability of VAM spores as reflected by percent VAM infection, spore production and plant growth parameters (Table 3.3.3.3).

### 3.3.4 Discussion

Lentil, maize and the sorghum-sudangrass hybrid supported growth and spore production by the VAM fungus *G. geosporum*. However, maize was the best crop under the growth conditions used in this study for producing the greatest number of *G. geosporum* spores per g of soil:sand mix. In contrast, Ferguson (1981) found no difference in *G. deserticola* spore production on seven host crops (peanut, asparagus, bell pepper, barley tomato, alfalfa and sudangrass) grown in autoclaved sand. Although sorghum is the most commonly used host crop for the production and maintenance of VAM inoculum (Kormanik et al. 1980; Hetrick and Bloom 1986), Bagyaraj and Manjunath (1980a) reported that sorghum was not as good a host as guinea grass for production of *G. fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe spores. I found that a sorghum-sudangrass hybrid was not as effective a host crop as maize for *G. geosporum* spore production. Struble and Skipper (1988) also found maize to be a better
Table 3.3.3.3 Effect of temperature and osmotic stress on the viability and (or) infectivity of *Glomus mosseae* spores as determined by percent VAM infection, spore production and growth of wheat and sorghum-sudangrass hybrid

<table>
<thead>
<tr>
<th>VAMa (°C)</th>
<th>Storage temp. (°C)</th>
<th>Osmotic stress</th>
<th>Plant dry weight (mg per pot)</th>
<th>Infection (%)</th>
<th>No. of spores (per100 g)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wheat Shoot</td>
<td>Root</td>
<td>Grain</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>1208b</td>
<td>753</td>
<td>750</td>
</tr>
<tr>
<td>yes</td>
<td>7</td>
<td>no</td>
<td>1570a</td>
<td>673</td>
<td>810</td>
</tr>
<tr>
<td>yes</td>
<td>25</td>
<td>no</td>
<td>1288b</td>
<td>735</td>
<td>740</td>
</tr>
<tr>
<td>yes</td>
<td>7</td>
<td>yes</td>
<td>1570a</td>
<td>803</td>
<td>825</td>
</tr>
</tbody>
</table>

LSD (0.05) 205 ns ns 497 ns 4.4 7.6 11 207

aTwo seedlings were inoculated with 150 spores, and plants grown in styrofoam cup containing 450 g soil:sand mix in a growth chamber.

bSpores were extracted from the entire cup and made to 100 mL in tap water. Numbers estimated by counting spores in two 20-mL aliquots.

cValues are means of four replications; numbers within a column followed by the same letter are not significantly different (P = 0.05).
Figure 3.3.3.4 Response of wheat plants to inoculation with *Glomus mosseae* spores stored at 7°C.
host than sorghum for production of *G. macrocarpum* Tul. and Tul., *G. clarioideum* Schenck and Smith and *G. etunicatum* Becker and Gerd. spores.

Spore production by *G. geosporum* grown on maize was related to the amount of VAM infected roots and the levels of nutrients available in the 2 and 6 kg soil:sand mix used to support crop growth. I found that nearly twice as many spores were obtained per g soil:sand when plants were grown in 2 kg pots compared to 6 kg pots. The root:shoot ratio of maize and the amount of VAM infected roots decreased when the soil volume was increased, which suggest that plants were not severely stressed for nutrients. In contrast, leaves and stems of plants grown in 2 kg pots exhibited symptoms of severe nutrient deficiency. This was related to the fact that these plants had to "mine" the soil for up to 45% of the nutrients (i.e., N and P) required for growth. This nutrient stress might explain the increased VAM infection, amount of VAM infected roots and spore production I observed in the 2 kg soil:sand system. Other studies (Ratnakaye et al. 1978; Douds and Schenck 1990; Sachay et al. 1991) also reported increased production of roots, root exudation, VAM infection and VAM spore production under N and P deficient conditions.

Although my study suggests VAM spore production was related to the total levels of available nutrients in the growth medium, total root production as influenced by the limiting space of the growth system might be important. For example, Ferguson (1981) reported that spore production by *G. deserticola* Trappe, Bloss and Menge on sorghum as host increased 90-fold when the pot volume (growth substrate) was increased 20-fold. However, on per gram basis spore production increased five times when pot volume was increased 20 times.

He attributed greater spore production (in the large soil volume) to increased root production. Total nutrient available for plant growth was not provided, and I can not
make a fair comparison to our study. However, Daft (1991) studied the effects of *G. mosseae* and different volumes of substrate on the performance of alfalfa and maize. He found that increasing the amount of P applied to *G. mosseae* inoculated maize plants grown in 1220 cm$^3$ and 5800 cm$^3$ pots decreased the percentage of VAM infection (approximately 37% in both pot sizes) and mycorrhizal dependency; VAM spore production was not assessed. Using his data I compared the percentage of infected maize roots for plants grown in 1220 cm$^3$ and 5800 cm$^3$ pots when supplied with approximately equal amount of P. Based on my calculations there were 87 mg of P in the 1220 cm$^3$ pots and 104 mg in the 5800 cm$^3$ pots; these levels were far in excess of that required for vegetative growth. In this comparison, the percentage of infected roots in 1220 cm$^3$ pots was lower (51%) compared to the roots in 5800 cm$^3$ pots (69%). Thus Daft's results support our conclusion that nutrient stress exerts more of an influence than substrate volume on VAM infection of maize roots, and presumably spore production.

Nevertheless, it would be interesting to assess the effects of increased nutrient levels (equivalent of 6 kg pots) on *G. geosporum* spore production when plant growth is limited by the confined space of 2 kg pots. Increased light intensity increase photosynthesis and possibly supply of carbohydrate to roots which enhance fungal growth and spore production.

In addition to soil nutrient levels, other factors may influence spore production by *G. geosporum*. For example, in my first experiment with *G. geosporum* (optimization study) 50% more spores were produced compared to the monospecific culture experiment. The maize plants in this optimization study were grown in a different growth chamber with ca. 48% more light intensity. Daft and El-Giahmi (1978) also observed an increase in mycorrhizal infection due to an increase in radiant energy, and Ferguson and Menge (1982) reported ca. 50% increase in spore production by *G. fasciculatum* on sorghum when the light intensity was increased by 42% (i.e., from 161 to 230 μE
m^{-2} s^{-1}). This increased in spore production due to increased light intensity was related to increase in carbohydrate production and root exudation.

Mycorrhizal spores are often used as inocula to study VAM-plant interactions. Spores are usually obtained from monospecific cultures by wet sieving and decanting, followed by sucrose density gradient centrifugation. Nevertheless, considerable amounts of organic detritus and soil particles remain mixed with spores even after density gradient centrifugation. I observed that the monospecific cultures of the three VAM species, *G. geosporum*, *G. mosseae*, *G. versiforme* contained spores of varying size classes, characteristic for each species. Furthermore, 62-86% of the spores of a particular species belonged to a narrow size range. Thus large numbers of VAM spores free of organic detritus may be obtained by using appropriate sieves of the desired size range. This may facilitate inoculant studies where VAM spores are required in large numbers.

Storage conditions are known to affect the viability of VAM spores. For example, Ferguson and Woodhead (1982) reported that whole pot cultures (containing spores, hyphae and infected roots) have been stored successfully for 4 years at 5°C with little loss in viability. Daft et al. (1987) found that increasing the storage temperature of *G. clarum* Nicol. and Schenck spores reduced intensity of infection in roots of alfalfa. I found that the viability of *G. mosseae* spores (as determined by percent VAM infection and plant growth parameters for wheat and the sorghum-sudangrass hybrid) in the monospecific cultures stored at 25°C was reduced compared to spores stored at 7°C. The reasons for the ineffectiveness of these spores stored at 25°C are not known. Spores of some VAM species undergo an innate dormancy period (Tommerup 1983). Furthermore, Gemma and Koske (1988) reported that dormancy of *Gi. gigantea* (Nicol. and Gerd.) Gerd. and Trappe spores was "released" by incubation at 5°C for 5 wk, whereas spores stored at 20-25°C for 5 wk were still dormant (as determined by their germination on water agar).
Safir et al. (1990) observed *ca. 60%* and *5%* germination (on water agar) of spores from a *G. mosseae* inoculum stored at -10° and 25 °C for 58 days, respectively. They attributed the enhanced spore germination to the breakage of innate dormancy and synchronization in germination of spores stored at -10°C. My results might be explained by synchronization of spore germination. However, Safir et al. (1990) did not observe increased germination of *G. mosseae* spores stored at 4°C, and additional studies are needed in this area. An alternative explanation might involve parasitism of *G. mosseae* spores stored at 25°C. For example, Daniels and Menge (1980) observed hyperparasitization of *G. fasciculatum* spores by two parasitic fungi, and Sylvia and Schenck (1983) found that germination of *G. clarum* spores was negatively correlated with the amount of parasitic fungi contaminating the spores. However, inoculum potential of spores at the time of storage at 25°C and at the time of inoculation of wheat and the sorghum-sudangrass hybrid was determined. For this reason it is difficult to indicate the cause of reduced infection in the two crops inoculated with *G. mosseae* spores stored at 25°C.

Exposure of *G. mosseae* spores to 20% and 60% sucrose solutions for 6-8 min during density gradient centrifugation had no adverse effect on their ability to infect wheat and the sorghum-sudangrass hybrid and promote plant growth. *Glomus mosseae* was chosen for this study because the spore wall is thinner than *G. geosporum* and *G. versiforme* (Schenck and Perez 1988). It was thought that thin walled spores might not withstand the osmotic stress exerted by sucrose solution. This suggests that the viability of *G. geosporum* and *G. versiforme* spores (which possess thick walls) will not be affected by osmotic stress.

Monospecific cultures of *G. geosporum*, *G. mosseae* and *G. versiforme* were produced with maize as host crop favorable conditions (Section 3.3.2.1) with maize.
*Glomus geosporum* produced the largest number of spores, reflecting either its inherent ability to produce more spores or a more favorable response than *G. mosseae* and *G. versiforme* to maize as a host crop. Hetrick and Bloom (1986) found that the trend for VAM spore production on five host crops [asparagus, marigold (*Tagetes erecta* L.), red clover, sudangrass and tomato] was: *G. macrocarpum* > *G. mosseae* > *G. fasciculatum*, except that *G. fasciculatum* produced more spores than *G. mosseae* on sorghum.

Furthermore, they observed that spore production of *G. fasciculatum* was significantly influenced by the host plant, whereas *G. macrocarpum* and *G. mosseae* were not. In my study, *G. geosporum* produced more than twice as many spores per g plant dry matter of maize and lentil plant than sorghum (Table 3.3.3.2). On maize as a host spore production was: *G. geosporum* > *G. versiforme* > *G. mosseae*. Spore production by *G. mosseae* and *G. versiforme* on lentil and the sorghum-sudangrass hybrid as hosts was not studied.

The ability of *G. geosporum* to produce large numbers of spores on maize suggests that this VAM is a good candidate for research where large amounts of spores are required and possibly for VAM inoculum production. For example, large number of spores could be used as inocula for horticultural crops or field studies at sites with reduced VAM propagule density.
3.4 Growth of lentil and wheat inoculated with *Glomus geosporum*, *Glomus mosseae* and *Glomus versiforme*

3.4.1 Introduction

Vesicular-arbuscular mycorrhizae (VAM) are an important group of soil fungi because they stimulate plant growth in agricultural soils with low levels of available P (Abbott and Robson 1981; Abbott and Robson 1984a; Schubert and Hayman 1986). Many different VAM species inhabit agricultural soils (Hall 1977; Schenck and Smith 1981; An et al. 1990; Johnson et al. 1991a), but their influence on crop growth may differ. For example, VAM inoculants differ in their ability to increase plant growth in sterile soils (Powell 1982; Jensen 1984a; Miller et al. 1985; Haas and Krikun 1985; Vierheilig and Ocampo 1991), and in natural soils in the presence of indigenous VAM (Abbott and Robson 1981; Medina et al. 1988). Other VAM species may depress host plant growth, either transiently or permanently depending upon the growth conditions (Bethlenfalvay et al. 1983; Koide 1985; Hung et al. 1990). Thus it is important to understand the relative contribution of a VAM species, either alone or in the presence of other VAM, to crop growth (Daft and Hogarth 1983; Koomen et al. 1987). This information may be useful for practical exploitation of VAM in crop production.

The reasons why one VAM species may be more effective than others in stimulating growth of host plants are not well understood. The performance of the VAM symbiosis is influenced by combinations of host-endophyte-environment (Bethlenfalvay et al. 1982). In some cases, effective VAM strains rapidly colonize and infect roots (Sanders et al. 1977), exhibit a greater production and faster spread of extramatrical hyphae, and a higher rate of P uptake (Graham et al. 1982a; Jakobsen et al. 1992). However, an effective strain under one set of growth conditions may not be effective under other conditions (Hayman and Tavares 1985; Medina et al. 1988). In order to exploit VAM in
crop production, it is necessary to assess the effects of indigenous VAM species on the growth of crops in specific agricultural soils. *Glomus geosporum, G. mosseae* and *G. versiforme* are widespread in soils of Saskatchewan (Section 3.1). In this study I assessed the effects of these three VAM species, either alone or in combination, on the growth of lentil and wheat under growth chamber conditions.

3.4.2 Materials and Methods

3.4.2.1 VAM species and inoculum preparation

Vesicular-arbuscular mycorrhizal spores were isolated from wheat field soils. *Glomus geosporum* and *G. mosseae* were from Outlook, Saskatchewan and *G. versiforme* from Semans, Saskatchewan, respectively (section 3.1). The Outlook (Bradwell association, sandy texture, 10 µg available P g⁻¹) and Semans (Weyburn association, loamy texture, 35 µg available P g⁻¹) soils are classified as Dark Brown Chernozems. Monospecific cultures of each VAM isolate were produced in an autoclaved soil:sand mix (1:1 w-w) using maize as the host crop in a growth chamber (see Section 3.3). At harvest, soil:root mixtures (i.e., monospecific inocula of infective VAM propagules) were air dried at 25°C for one week and stored at 7°C for six weeks. Spores of *G. geosporum* (106-250 µm), *G. mosseae* (106-250 µm) and *G. versiforme* (53-120 µm) were obtained by wet sieving the monospecific VAM cultures (Gerdeman and Nicolson 1963); debris was removed by a sucrose density gradient (20% and 60% w/v) centrifugation (Daniels and Skipper 1982). After density gradient centrifugation, considerable amounts of hyphal fragments still contaminated the VAM spore preparation. The entire VAM spore mixture (in a 15 mL centrifuge tube) was vortexed and spores allowed to settle before hyphal fragments were decanted off. This step was repeated 3-4 times; *G. geosporum* and *G. versiforme* spores appeared to be free of hyphae whereas
G. mosseae and spores still contained small amounts of hyphae (shown by arrow in Fig. 3.3.3.2 in Section 3.3.3).

3.4.2.2 Effect of G. geosporum, G. mosseae and G. versiforme on lentil

Five treatments were assessed: (1) G. geosporum, (2) G. mosseae, (3) G. geosporum + G. mosseae, (4) G. versiforme and (5) an uninoculated control. Lentil seeds were surface sterilized by soaking in 70% (v/v) alcohol for 1 min, 1.2% (w/v) sodium hypochlorite for 5 mins, and washed 8 times with sterile distilled water. Seeds were aseptically germinated on 0.3% Trypticase soy agar (0.3% Trypticase soy broth, 1.5% agar). Two 5-day-old seedlings were transferred to pots and inoculated with 100 VAM spores by a funnel technique (Fig. 3.4.2.1) (Menge and Timmer 1982). Co-inoculated plants received 50 spores each of G. geosporum and G. mosseae.

Uninoculated control plants received 5 mL of a water filtrate obtained by passing a mixed suspension of G. geosporum and G. mosseae spores through a 1.2 μm millipore filter (cellulose nitrate membrane filter, diam. 25-mm, Cat no. RAWP 025 00, Millipore corporation, Belford, MA 01730).

3.4.2.2.1 Plant growth conditions

Plants were grown in 15 cm plastic pots containing 2 kg of a soil:sand mix. Outlook soil [pH 7.6 and (μg g⁻¹) NO₃-N, 43; available P, 10; available K, 317; SO₄-S, 12.4; Cu, 0.67; Fe, 10.2; Zn, 1.2; Mn 12.9] was mixed with sand (silica sand grade #7, Target Product Limited, Calgary, Edmonton, Canada) at 1:1 ratio. The soil:sand mix was amended with 50 mL of a modified Hoagland solution kg⁻¹ mix to compensate for original nutrient levels (see section 3.1.2.4) and autoclaved (at 121°C for 1h) to eliminate indigenous VAM. The autoclaved soil:sand mix had a pH of 7.6 and contained (μg g⁻¹) NO₃-N, 30; available P, 10; available K, 320; SO₄-S, 15.6; Cu, 0.5; Fe, 7.5; Zn, 1.4; and Mn, 75.0.
Figure 3.4.2.1 Funnel technique used to inoculate seedlings of wheat and lentil.
Pots were placed in a growth chamber (photosynthetic irradiance 250-300 μE m\(^{-2}\) s\(^{-1}\)) with a 16 h light (26°C) : 8 h dark (20°C) cycle. Pots were watered daily with sterile distilled water to maintain the soil:sand mix at 70% moisture holding capacity (ca. 60 kPa moisture tension). Each pot received 50 mL of a modified Hoagland (minus P) solution (Section 3.1) on the 3rd, 4th and 5th week and 50 mL of 75 ppm N as KNO\(_3\) solution (540 ppm) on 6th, 7th and 8th week. Lentil was not inoculated with *Rhizobium*.

**3.4.2.2 Plant growth parameters and VAM infection**

Lentil plants from 4 replicate pots were harvested at 31, 56 and 100 DAP. The three harvests correspond to initiation of flowering, pod setting and completion of maturity of pods, respectively. The comparative effects of the three VAM species were assessed by plant shoot and root dry weight (54°C at 72 h) at each sampling interval, grain yield at final harvest, and shoot and grain P content. Senesced leaves were also collected from each pot, oven dried (72 h at 54°C), weighed and included in the total shoot dry weight of lentil at the various sampling intervals.

To assess VAM infection, the whole root system of each replicate sample was recovered, cut into 1 cm long pieces, mixed and a representative 3 g (fresh weight) sample cleared and stained in lactoglycerol trypan blue (Phillips and Hayman 1970). Infection (percent infected root length) was measured using the grid-line intersect method (Giovannetti and Mosse 1980). The number of intersections with arbuscular infection was also recorded.

**3.4.2.3 Nutrient content**

Phosphorus in shoots (leaves plus stems) and seeds were determined by following the procedure described earlier (Section 3.3.2.1.3).
3.4.2.4 Recovery of VAM spores

Spores of *G. geosporum*, *G. mosseae* (106-250 µM) and *G. versiforme* (44-120 µM) were recovered from the entire 2 kg soil:sand mix at the final harvest by wet sieving and density gradient centrifugation (see above). The spore suspension (recovered from the entire pot) was made up to 100 mL with distilled water and the number of spores in two 5-mL sub samples determined using a stereomicroscope (47×).

3.4.2.3 Effect of *G. geosporum* and *G. mosseae* on wheat

Four treatments were assessed: (A) *G. geosporum*, (2) *G. mosseae*, (3) *G. geosporum* + *G. mosseae*, and (4) an uninoculated control. The experiment was set up as for lentil, except that wheat plants did not receive KNO₃ solution (Section 3.4.2.2.1) and were harvested at 35 (completion of tillering), 48 (panicle emergence) and 83 (maturity) DAP. In addition to the parameters recorded for lentil, the effect of inoculation on plant height and number of tillers at 83 DAP was also assessed.

3.4.2.4 Statistics

Data were analyzed by separate one way analysis of variance. When F values were significant, the Least Significant Difference test was used to separate the means (Little and Hills 1979). The treatment differences for all parameters are reported at $P = 0.05$. 
3.4.3 Results

3.4.3.1 Effect of *G. geosporum*, *G. mosseae* and *G. versiforme* on lentil

3.4.3.1.1 Plant shoot and grain yield

The three VAM inoculants increased shoot biomass of lentil compared to the uninoculated control (Table 3.4.3.1). The positive growth response became evident at different sampling intervals depending upon the VAM isolates. For example, *G. geosporum* and *G. versiforme* increased shoot biomass at 31 and 56 DAP onward, respectively, whereas *G. mosseae* increased shoot biomass at 100 DAP. *Glomus geosporum* was the most effective VAM inoculant at increasing shoot biomass of lentil. All VAM inoculants increased grain yield. Inoculation with *Glomus geosporum* resulted in highest increase (57%) in grain yield followed by *G. versiforme* (29%) and *G. mosseae* (26%). The results obtained by co-inoculation with the *G. geosporum* and *G. mosseae* is presented under section 3.4.3.4

3.4.3.1.2 Root weight and VAM infection

VAM inoculants had no effect on root biomass until 56 DAP after which biomass was significantly reduced compared to the uninoculated control. The reduction of root biomass was highest in *G. geosporum* inoculated lentil plants (Table 3.4.3.1). The percentage of root length colonized by the three VAM fungi was different at different sampling intervals (Fig. 3.4.3.1). *Glomus geosporum* achieved the highest root colonization at all harvests followed by *G. versiforme* and *G. mosseae*. *Glomus geosporum* also formed more arbuscules. The levels of arbuscular infection by individual VAM species (Fig. 3.4.3.1) followed a trend similar to the total VAM infection. The shoot biomass of lentil inoculated with the different VAM was significantly correlated with the percent VAM infected roots at 31 and 56 DAP (Fig. 3.4.3.2). However, shoot
Table 3.4.3.1 Growth and yield of lentil inoculated with *Glomus geosporum*, *Glomus mosseae* or *Glomus versiforme* spores and grown in a growth chamber

<table>
<thead>
<tr>
<th>Inoculant(^a)</th>
<th>Shoot dry weight (mg per pot)</th>
<th>Root dry weight (mg per pot)</th>
<th>Grain yield (mg per pot)</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>523b(^b) 7860d 7860e</td>
<td>424 3687a 3690a</td>
<td>2799d 100</td>
<td></td>
</tr>
<tr>
<td><em>G. geosporum</em></td>
<td>735a 12310a 10800a</td>
<td>429 3004b 2214c</td>
<td>4401a 157</td>
<td></td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>585b 7888d 8451d</td>
<td>402 3121b 3132b</td>
<td>3529c 126</td>
<td></td>
</tr>
<tr>
<td><em>G. versiforme</em></td>
<td>533b 9799c 9098cb</td>
<td>370 3262b 3074b</td>
<td>3629c 129</td>
<td></td>
</tr>
<tr>
<td><em>G. geosporum</em> + <em>G. mosseae</em></td>
<td>668a 11090b 9167b</td>
<td>426 2935b 2386c</td>
<td>3962b 141</td>
<td></td>
</tr>
<tr>
<td><strong>LSD(_{0.05})</strong></td>
<td>128 1810 445</td>
<td>ns 432 225</td>
<td>320 -</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Two seedlings were inoculated with 100 spores by a funnel technique and grown in a 15 cm pot containing 2 kg sterilized soil:sand mix; co-inoculated seedlings received 50 spores of each species.

\(^b\)Values are means of 4 replications; numbers within a column followed by the same letter are not statistically \((P = 0.05)\) different as determined by the least significant difference test.
Figure 3.4.3.1 Effect of inoculation with *Glomus geosporum* (△), *Glomus mosseae* (○), *Glomus geosporum* + *Glomus mosseae* (●) and *Glomus versiforme* (□) on the percentage of (A) total VAM infection and (B) arbuscular infection in lentil roots at 3 harvests. Vertical lines represent least significant differences between treatment means (P = 0.05).
Figure 3.4.3.2 Relationship between shoot dry weight and percentage of VAM infection in lentil roots at (A) 31 and (B) 56 days after planting. **, statistically significant at $P = 0.01$. 

\[
R^2 = 0.650** \\
\]

\[
R^2 = 0.802** \\
\]
biomass at 100 DAP and grain yield were not significantly correlated with the percent
VAM infected roots at 100 DAP.

3.4.3.1.3 P content of shoots and grain

All three VAM species increased the P content of lentil shoots at 56 DAP (except for
*G. mosseae*) and of grain at 100 DAP compared to the uninoculated control (Table
3.4.3.2). However, *G. geosporum* caused the greatest increase in the P content of lentil
shoot and grain. The P content of shoot and grain of co-inoculated lentil was similar to
that of *G. geosporum* inoculated plant.

3.4.3.2 Effect of *G. geosporum* and *G. mosseae* on wheat

3.4.3.2.1 Tiller numbers and plant height

Plant growth parameters differed depending on the VAM inoculant (Fig. 3.4.3.3
and Table 3.4.3.3). For example, *G. geosporum* either alone or co-inoculated with *G.
mosseae* significantly increased the number of tillers compared to the control and *G.
mosseae* inoculated plants (Fig. 3.4.3.4 and Table 3.4.3.3). Inoculation with *G.
mosseae*, however, caused a significant increase in plant height by 83 DAP.

3.4.3.2.2 Plant shoot and grain yield

*Glomus geosporum* stimulated early shoot growth of wheat, whereas *G. mosseae*
depressed shoot growth (Table 3.4.3.3 and Fig. 3.4.3.3). These effects were transitory
as no difference in shoot dry weight was detected at harvest (83 DAP). However, *G.
geosporum* increased grain yield by 12%.
Table 3.4.3.2 Phosphorus content of shoots and grain of lentil inoculated with *Glomus geosporum*, *Glomus mosseae* or *Glomus versiforme* and grown in a growth chamber.

<table>
<thead>
<tr>
<th>Inoculant</th>
<th>P (mg g⁻¹)</th>
<th>Shoot</th>
<th>Grain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>31 DAP b</td>
<td>56 DAP</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.76</td>
<td>2.67d c</td>
<td>3.77d</td>
</tr>
<tr>
<td><em>G. geosporum</em></td>
<td>2.93</td>
<td>3.47a</td>
<td>5.22a</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>2.68</td>
<td>2.90bd</td>
<td>4.23c</td>
</tr>
<tr>
<td><em>G. versiforme</em></td>
<td>2.81</td>
<td>3.04bc</td>
<td>4.52bc</td>
</tr>
<tr>
<td><em>G. geosporum</em> + <em>G. mosseae</em></td>
<td>2.80</td>
<td>3.27ac</td>
<td>4.89ab</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>ns</td>
<td>0.30</td>
<td>0.40</td>
</tr>
</tbody>
</table>

aTwo seedlings were inoculated with 100 spores by a funnel technique and grown in 15 cm pot containing 2 kg sterilized soil:sand mix; co-inoculated seedlings received 50 spores of each species.

bDAP, days after planting.

cValues are means of 4 replications; numbers within a column followed by the same letter are not statistically different (P = 0.05).
Figure 3.4.3.3 Effect of *Glomus geosporum* and *Glomus mosseae* on the growth of wheat at 48 days after planting. Note early panicle emergence (arrows) in *Glomus geosporum* inoculated plants.
Figure 3.4.3.4 Effect of *Glomus geosporum* and *Glomus mosseae* on the growth of wheat at 83 days after planting. Note tiller numbers (white arrows) in *Glomus geosporum* inoculated plants and height of *Glomus mosseae* inoculated plants.
Table 3.4.3.3  Growth and yield of wheat inoculated with *Glomus geosporum* or *Glomus mosseae* spores and grown in a growth chamber

<table>
<thead>
<tr>
<th>Inoculant&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
<th>Days after planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot dry weight</td>
<td>Root dry weight</td>
<td>Shoot dry weight</td>
<td>Root dry weight</td>
<td>Shoot dry weight</td>
<td>Root dry weight</td>
<td>Shoot dry weight</td>
<td>Root dry weight</td>
<td>Shoot dry weight</td>
<td>Root dry weight</td>
<td>Shoot dry weight</td>
<td>Root dry weight</td>
</tr>
<tr>
<td></td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
<td>(mg per pot)</td>
</tr>
<tr>
<td>Control</td>
<td>4175&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9052&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8590</td>
<td>1676&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2627</td>
<td>2629&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. geosporum</em></td>
<td>4205&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9616a</td>
<td>9053</td>
<td>1718&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2669</td>
<td>2101&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5640&lt;sup&gt;a&lt;/sup&gt;</td>
<td>112</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>3793&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8354&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9020</td>
<td>1520&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2374</td>
<td>2544&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4820&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. geosporum</em> +</td>
<td>4545&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9729&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8937</td>
<td>1968&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2577</td>
<td>2327&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>6.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5680&lt;sup&gt;a&lt;/sup&gt;</td>
<td>113</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD&lt;sub&gt;(0.05)&lt;/sub&gt;</td>
<td>258</td>
<td>453</td>
<td>ns</td>
<td>293</td>
<td>ns</td>
<td>227</td>
<td>0.65</td>
<td>4.8</td>
<td>228</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Two seedlings were inoculated with 100 spores by a funnel technique and grown in 15 cm pot containing 2 kg sterilized soil:sand mix; co-inoculated seedlings received 50 spores of each species.

<sup>b</sup> Values are means of 4 replications; numbers within a column followed by the same letter are not statistically different ($P = 0.05$) as determined by the least significant difference test.
3.4.3.2.3 Root weight and VAM infection

There was no consistent effect of either VAM inoculant on root dry weight. The *G. geosporum* inoculant had no effect on root growth at 35 and 48 DAP (Table 3.4.3.1) but had significantly reduced root weight at harvest (i.e. 83 DAP). Root growth of co-inoculated wheat plant was similar to that of *G. mosseae* inoculated plants and the uninoculated control except at 83 DAP. The percentage of root length infected by either VAM isolate was not statistically different at 35 and 48 DAP (Fig. 3.4.3.5). However, roots of *G. geosporum*-inoculated plants exhibited significantly higher levels of infection than that of *G. mosseae* inoculated plants at the final harvest (83 DAP). Furthermore, *G. geosporum* infected roots contained more arbuscules than *G. mosseae* infected roots at all sampling intervals (Fig. 3.4.3.5).

3.4.3.2.4 P content of shoots and grain

*Glomus geosporum* significantly increased the P content of wheat shoots at 35 and 48 DAP, and of the grain at the final harvest (83 DAP). In contrast *G. mosseae* only increased the P content of shoots at 35 DAP, after which it had no effect on P content of wheat shoot and grain (Table 3.4.3.4).

3.4.3.3 Infection morphology of VAM in wheat and lentil

The infection of wheat and lentil roots by *G. geosporum* was dominated by arbuscules, whereas the *G. mosseae* infected segments of wheat and lentil contained fewer arbuscules (Fig. 3.4.3.6 and 3.4.3.7). No vesicles were produced by *G. geosporum* in lentil and wheat roots and *G. mosseae* produced only a few vesicles in lentil roots. The infection of lentil roots by *G. versiforme* was dominated by large numbers of vesicles.
Figure 3.4.3.5 Effect of inoculation with *Glomus geosporum* (△), *Glomus mosseae* (○), and *Glomus geosporum* + *Glomus mosseae* (●) on the percentage of (A) total VAM infection and (B) arbuscular infection in wheat roots at 3 harvests. Vertical lines represent least significant differences ($P = 0.05$) between treatment means.
Table 3.4.3.4 Phosphorus content of shoots and grain of wheat inoculated with *Glomus geosporum* or *Glomus mosseae* and grown in a growth chamber

<table>
<thead>
<tr>
<th>Inoculant&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Phosphorus (mg g&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>35 DAP&lt;sup&gt;b&lt;/sup&gt;</th>
<th>48 DAP</th>
<th>Grain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>2.56&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.38&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>G. geosporum</em></td>
<td></td>
<td>2.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td></td>
<td>2.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.41&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>G. geosporum</em> +&lt;sup&gt;+&lt;/sup&gt; <em>G. mosseae</em></td>
<td></td>
<td>2.71&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;(0.05)&lt;/sub&gt;</td>
<td></td>
<td>0.28</td>
<td>0.37</td>
<td>0.21</td>
</tr>
</tbody>
</table>

<sup>a</sup>Two seedlings were inoculated with 100 spores by a funnel technique and grown in a 15 cm pot containing 2 kg sterilized soil:sand mix; co-inoculated seedlings received 50 spores of each species.

<sup>b</sup>DAP, days after planting.

<sup>c</sup>Values are means of 4 replications; numbers within a column followed by the same letter are not statistically different (P = 0.05).
Figure 3.4.3.6 Photomicrographs of 56 day old lentil root showing infection morphology of *Glomus geosporum*, *Glomus mosseae* and *Glomus versiforme* inside lentil roots. (A) Abundant arbuscule (ar) production by *Glomus geosporum*, (B) Few arbuscules produced by *Glomus mosseae*, (C) *Glomus mosseae* produces few intraradical vesicles (ve), (D) Arbuscular infection by *Glomus versiforme*, (E) and (F) Abundant vesicle production by *Glomus versiforme*. 
Figure 3.4.3.7 Photomicrographs of 48 day old wheat root showing infection morphology (A) Abundant arbuscule (ar) production by *Glomus geosporum* and (B) Infection morphology of *Glomus mosseae* is characterized by small arbuscules and intraradical hyphae (h).
3.4.3.4 Effect of co-inoculation with *G. geosporum* and *G. mosseae* on lentil and wheat

The shoot biomass of co-inoculated lentil was lower than *G. geosporum*-inoculated plants and higher than *G. mosseae*-inoculated plants (Table 3.4.3.1). The pattern of VAM infection in co-inoculated roots was similar to *G. geosporum* inoculated roots (Figs. 3.4.3.1 and 3.4.3.5). Wheat plants co-inoculated with *G. geosporum* + *G. mosseae* tended to grow better and produce more biomass compared to plants inoculated with *G. geosporum* or *G. mosseae*. This observation was not statistically significant except at 35 DAP (Table 3.4.3.3) when shoot weight of co-inoculated plants was significantly higher than that of the other treatments. Furthermore, the relative number of spores produced in the lentil and wheat rhizospheres by the two VAM species, either alone or when co-inoculated, indicated competition between the two isolates (Table 3.4.3.5). For example, *G. geosporum* produced twice as many spores as *G. mosseae* in the rhizospheres of wheat and lentil plants when inoculated alone. On the other hand, co-inoculation resulted in approximately a 2- and 3-fold reduction in spore numbers for *G. mosseae* in lentil and wheat rhizospheres, respectively, but only a slight reduction for *G. geosporum* in both crops.

3.4.4 Discussion

The vesicular-arbuscular mycorrhizae *G. geosporum*, *G. mosseae* and *G. versiforme* are widespread in Saskatchewan soils and co-exist in the wheat field soils (Section 3.1). However, these VAM species differed in their ability to infect, and affect, the growth of lentil and wheat grown in a sterile substrate. Both crops varied in the type and magnitude of their response to inoculation with these VAM species. For example, *G. geosporum* and *G. mosseae* both produced similar levels of infection in wheat roots, but only *G. geosporum* increased the growth and yield of wheat. *Glomus mosseae*, on the
Saskatchewan (Saskatchewan Soil Testing Laboratory, University of Saskatchewan). I found that VAM inoculation increased the P content of lentil shoots and grain, and the P uptake followed a trend similar to percentage of root length colonized i.e., \textit{G. geosporum} > \textit{G. versiforme} > \textit{G. mosseae}. Hung et al. (1990) also observed a positive correlation between the P content of sweet potato shoots and infected root length for three VAM isolates. Such relationships are not unexpected because they reflect both the importance of VAM to P uptake by plants and the effect of an adequate P supply on plant growth.

My results suggest some VAM exhibit a degree of host specificity in terms of their relative benefits to the host plant. For example, \textit{G. geosporum} increased the grain yield of wheat 12\% whereas a 56\% increase was noted for lentil. \textit{Glomus mosseae} had no effect on wheat yield, but increased the yield of lentil by 29\%. The specificity was also reflected in the number of VAM spores produced in the rhizospheres of these hosts. \textit{Glomus geosporum} produced twice as many spores as \textit{G. mosseae} regardless of the host. Furthermore, each of the VAM isolates produced 3-4 fold more spores in the lentil than in the wheat pots.

The difference in the relative efficacy of \textit{G. geosporum} and \textit{G. mosseae} might be explained by functional differences at the level of the host-fungus interface. \textit{Glomus geosporum} produced a significantly higher level of arbuscular infection in wheat and lentil roots compared to \textit{G. mosseae}. Since arbuscules are the most important structures that function in nutrient transfer between the host and fungus, the number of arbuscules produced by a VAM may reflect its relative benefit to the host crop. It is known that arbuscule production by a VAM species may be reduced considerably by increasing the amount of P applied (Braunberger et al. 1991; Clapperton and Reid 1992), but there is no information on arbuscule production by different VAM species in the same host or the relation between arbuscule levels and plant growth promoting ability of different VAM.
The presence of more than one VAM in the growth substrate may lead to competition between endophytes (Daft and Hogarth 1983; Wilson and Trinick 1983; Wilson 1984), and possibly lead to dual (or multiple) infections. Evidence for competition between specific VAM pairs is usually obtained by comparing the growth of a host plant inoculated with one VAM species alone and in combination with another. I found that co-inoculation of lentil and wheat with *G. geosporum* and *G. mosseae* either had no effect or slightly reduced the efficacy of the most beneficial VAM, *G. geosporum*. The more beneficial *G. geosporum* was for a host, the greater the impact of *G. mosseae*. For example, *G. mosseae* reduced the efficacy of *G. geosporum* by 16% when co-inoculated on lentil, but had no effect when the host was wheat. Similarly, Koomen et al. (1987) found that *G. macrocarpum* (one of four VAM tested) had no effect on the growth of clover, nor did this species affect the growth enhancing ability of the other three VAM when co-inoculated on clover. However, they found that the beneficial effect of *G. mosseae* on clover was greatly reduced by co-inoculation with the ineffective *Glomus* E3.

Precise methods for evaluating competition between VAM species should be based on determining the relative proportion of infected root area occupied by each individual VAM when co-inoculated to a host plant. However, methods to distinguish anatomical structures produced by different VAM inside roots are relatively scarce (Abbott and Robson 1978; Wilson 1984) and it is difficult to draw firm conclusions about competition between VAM. Daft and Hogarth (1983) used VAM spore production, when inoculated alone or in combination with other VAM, as a measure of competition. They found spore production by *G. clarum* in on maize was greater than that of *G. caledonium*, *G. geosporum* or *G. mosseae*. However, spores produced by each individual VAM were greatly reduced when co-inoculated with other VAM. Spore production by *G. clarum* was affected least by co-inoculation indicating this VAM was more competitive. Based on spore production, I found that *G. geosporum* was more competitive than *G. mosseae*.
when co-inoculated to lentil and wheat. *Glomus geosporum* produced ca. 2000 and 500 spores per 100 g substrate, respectively in lentil and wheat pots. This was approximately 2-3 fold more spores than produced by *G. mosseae*. When co-inoculated, there was 15-19% reduction in spore production by *G. geosporum* versus a 50 to 70% decrease in spore production by *G. mosseae*.

The positive response of crops to VAM inoculation in the presence of indigenous VAM is an indication that indigenous VAM may be ineffective or less competitive (McGonigle 1988). My study shows that three VAM species (*G. geosporum*, *G. mosseae* and *G. versiforme*) which co-existed in Saskatchewan field soils differed in their competitiveness and contribution to the growth of lentil and wheat. *Glomus geosporum* was a very competitive and a highly effective VAM for lentil and wheat.
4 GENERAL DISCUSSION AND CONCLUSIONS

Vesicular-arbuscular mycorrhizal (VAM) symbiosis in the plant kingdom is a very ancient phenomenon. However, it is only in the last 25 years that research has advanced our understanding of this plant-fungus association. With this advancement have come new questions concerning complex aspects of VAM-plant interactions. For example, how do changing plant growth conditions influence (i) VAM function and activity, or (ii) competition between different VAM species for infection sites on hosts. Other important questions deal with issues such as the population dynamics and activity of VAM in relation to changes in the soil physico-chemical and biological environment, perhaps in response to cultivation practices of agroecosystems or natural plant community succession.

My thesis research assessed the occurrence and significance of VAM in Saskatchewan soils and field crops. The first part was a field survey of the types of VAM fungi in soils and their population dynamics and activity as influenced by cropping practices. This study was followed by a series of growth chamber experiments designed to isolate VAM species from soils, develop methods to produce monospecific cultures of VAM isolates, and assess the effectiveness of different species either alone or in competition with one another to enhance the growth of wheat and lentil.

The field survey was divided into two parts. First, I assessed VAM spore populations in soils and VAM infection in wheat and lentil at sites across four soil zones. The second part evaluated the impact of short-term crop rotations on VAM population dynamics (i.e., spore numbers) in soils and VAM infection of subsequent wheat and barley crops. This type of information might provide insight into how changing plant growth conditions influence VAM function and activity. I found that the number of VAM spores in Saskatchewan agricultural soils ranged from 78-272 per 100 g soil in the 1988
study and 410-2113 per 100 g soil in the 1989 study. Kucey and Paul (1983) also reported that VAM spores in Saskatchewan soils ranged from 41-332 per 100 g soil. These numbers were similar to levels detected in other agricultural soils (Abbott and Robson 1977b; Hayman and Stovold 1979; Malibari et al. 1988).

In addition to VAM spore numbers, I also determined the VAM infection levels of wheat and lentil grown in these soils. Wheat and lentil crops were readily infected by the indigenous VAM, but the infection patterns were different. In general, lentil exhibited higher levels of infection compared to wheat. The higher percentage of VAM infection in lentil roots compared to wheat roots may indicate more benefits of VAM to lentil. However, using the percentage of VAM infection to compare benefits of VAM to crops is subject to criticism (Jakobsen and Nielsen 1983; Abbott and Robson 1991a). Many authors (Fitter 1985; Abbott and Robson 1991a) suggest that the total VAM infected root length of a crop is a better indication of VAM benefits. It is well known that the rooting patterns of crops differ. Thus a coarse rooted crop may exhibit a high percentage of VAM infected roots, but the total root length infected with VAM may be less than that for a crop with fine roots which exhibits a low percentage of VAM infection. Unfortunately, it was not possible to determine the total VAM infected root length for crops in my field study. However, growth chamber studies show that the total VAM infected root length of wheat in a nonsterile soil is greater than lentil (F. Walley, personal communication). However, the relationship between the total VAM infected root length and growth response of different crops has received little attention (Jakobsen and Nielsen 1983).

The pattern of VAM infection in wheat and lentil during the growing season may be related to the function of VAM. The VAM infection in wheat became well established by the tillering stage, increased until panicle emergence, and then tended to decline. In contrast, lentil exhibited an initial rapid infection that lasted until the flowering stage, followed by a plateau until maturity. My results corroborate the three phase pattern of
The higher VAM infection in lentil during the early part of growth may be an indication of a greater P requirement. The constant level of VAM infection in lentil from flowering to maturity may reflect both the indeterminate growth of lentil and (or) a high P requirement. Thus lentil may benefit more from the VAM symbiosis than wheat.

The level of VAM infection in wheat and lentil varied among sites. Many other studies have recorded variation in VAM infection from site to site (Abbott and Robson 1991a). Based on my results and information in the literature, questions arise as to why these variations in the levels of VAM infection occur between sites? Do these differences indicate that VAM fungi have a significant impact on field grown crops? Based on my results for VAM infection of wheat at different sites, I believe that the benefits from VAM varied depending on the field site, with the maximum benefit occurring at sites with the highest level of infection. Although my study sites were located along a gradient of increasing soil moisture and decreasing soil temperature, there was no apparent trend of VAM infection in crops at these sites. Therefore, the VAM infection of a crop at different sites may reflect the complex interaction of climatic parameters, soil properties and the specific VAM community of a site. Hence it may be difficult to single out any particular factor as a cause of variation in VAM infection. This may be the reason why, in most studies (Hayman et al. 1976; Hayman 1982a,b), soil fertility of the study sites was not related to the VAM infection.

Phosphorus is the soil nutrient most important in determining the benefit of VAM to the host crops in growth chamber (Mosse 1973a; Hayman and Tavares 1976; Bethlenfalvay et al. 1982) and field studies (Black and Tinker 1979; Fitter 1985; McGonigle 1988). High P levels can reduce the benefits of VAM to crops (Mosse 1973a), because plants growing in a high P soil can absorb P adequately, and thus colonization of roots by VAM remains low. I expected to obtain a negative correlation
between available soil P and the level of VAM infection in wheat grown at different sites. However, no correlation was obtained between VAM infection and available soil P at different sites indicating that available P was not a factor in controlling VAM infection of wheat. An alternative reason for the observed variation in the VAM infection in wheat at different sites in Saskatchewan may be the number and type of VAM species present in the different soils.

Hayman et al. (1982a) and Abbott and Robson (1991b) suggested that natural variability of VAM propagule distribution may be associated with site to site variation in VAM infection. However, limited knowledge (Abbott and Robson 1982a; Trinick 1984) on the infection morphology of different VAM species in host roots makes it difficult to determine the presence of a specific VAM within the indigenous community at a particular study site. Nevertheless, I observed that the infected wheat roots at different sites contained vesicles of different shapes, indicating infection by different VAM species. This observation led me to determine the number of different VAM species present in wheat field soils at different study sites. In general, the VAM community in a soil is determined by identification of the spores extracted directly from that soil. In my study, however, I first enriched the indigenous VAM propagules of field soils on maize and then determined the type and number of VAM species present in those soils. The reason I used this enrichment technique is because parasitization of spores in field soils (Ross and Ruttencutter 1977; Daniels and Menge 1980) makes their identification difficult. The enrichment technique I used allowed isolation of fresh VAM spores which facilitated identification.

Seven VAM species were isolated from six soils. The size of the VAM community varied in these six soils with the highest number (at least six species) occurring at the Outlook site and the fewest (at least three species) occurring at the Glenavon site. *Glomus* spp. were the most common VAM in Saskatchewan soils. However, the actual
size of the VAM community in the field soils used in my study may not be exactly the same as the number detected after enrichment of the indigenous VAM on maize. It is possible that (i) other VAM exist in Saskatchewan soils but did not grow in association with maize, or (ii) the VAM detected were not the most abundant but rather stimulated by maize. This is a weakness in this part of my study. Nevertheless, my study confirms the occurrence of at least seven VAM species in Saskatchewan soils. Based on these results I propose that the observed variation in VAM infection of wheat at different sites was due to differences in the size and type of the VAM community at a specific site.

The fact that different VAM species occurred in a particular soil raised several important questions which needed to be addressed. Are different VAM species from a particular site equally infective? Do these indigenous VAM in Saskatchewan soils differ in their ability to increase growth of field grown crops? Does the presence of more than one VAM species in a soil lead to competition between them? The answer to these questions requires a knowledge of how individual and mixtures of VAM influence crop growth. This required development of monospecific cultures of my VAM isolates.

Three VAM, *G. geosporum*, *G. mosseae* and *G. versiforme* were selected for testing their effectiveness on wheat and lentil. Two were the most common VAM detected in soils at all study sites. Furthermore, they produced large-sized spores (106-300 μm) which were easy to handle. *Glomus versiforme* was selected because it was much smaller (44-106 μm) compared to the other two species. Since VAM spore production is affected by a number of factors such as host crops, type of growth substrate, volume and nutrient regimes of a growth substrate, and light intensity (Menge 1984; Hetrick and Bloom 1986; Struble and Skipper 1988; Coltman et al. 1988), an initial growth chamber experiment was conducted to optimize VAM spore production prior to the testing of efficacy of these VAM. This study also was needed to develop a protocol to obtain a large number of spores for inocula.
Initially three hosts, maize, lentil and a sorghum-sudangrass hybrid were evaluated for their ability to support the growth and production of spores by *G. geosporum*. Two soil volumes (i.e., 2 kg and 6 kg soil:sand mix) were used to grow the host crops. Maize was the best crop for producing the greatest number of *G. geosporum* spores per g of soil:sand mix. Spore production by *G. geosporum* grown on maize was related to the amount of VAM infected roots produced by maize in 2 kg soil:sand mix, and also to the levels of nutrients available in the 2 and 6 kg soil:sand mix used to support crop growth. Nearly twice as many spores were obtained per g soil:sand when plants were grown in 2 kg pots compared to 6 kg pots. In contrast to my results, Ferguson (1981) increased spore production by *G. deserticola* on a sorghum host by increasing pot volume (growth substrate). In his study, spore production per gram of substrate increased 5-fold when pot volume was increased 20-fold. He attributed this greater spore production to increased root production. I obtained more spores by growing maize with very limited N and P levels in small pots (2 kg capacity). This result indicated that severe nutrient stress in a limited soil volume exerted a strong influence on spore production. This may be related to the observed increase in the root:shoot ratio, or possibly to an effect on root exudates. It would be interesting, therefore, to assess *G. geosporum* spore production when plant growth is limited by the confined space of 2 kg pots, but with unlimited nutrients (e.g., nutrient supply equivalent to that of 6 kg pots). Under these conditions the maize plants will probably grow better and produce less spores. Such an experiment would confirm that nutrient stress led to higher spore production. Future studies should also assess whether root exudates of maize plants are related to spore production.

Monospecific cultures of *G. geosporum, G. mosseae* and *G. versiforme* were produced under optimal conditions with maize. *Glomus geosporum* produced the largest number of spores, reflecting either its inherent ability to produce more spores or a more favorable response than *G. mosseae* and *G. versiforme* to maize as a host crop. Hetrick
and Bloom (1986) studied spore production by three VAM on five host crops and observed that spore production by *G. fasciculatum* was significantly influenced by the host plant, whereas *G. macrocarpum* and *G. mosseae* were not. I observed that, with maize as a host, spore production was: *G. geosporum* > *G. versiforme* > *G. mosseae*. I also observed that the size of the spores produced by these VAM species in monospecific cultures varied. Although spores of each VAM species were distributed over a wide size range (and many spore size categories existed for each species), 62 to 86% of the spores in the monospecific culture of a particular species belonged to a narrow size range. This observation allowed recovery of spores with less associated organic detritus by using appropriate sieves.

The effects of monospecific inocula of *G. geosporum*, *G. mosseae* and *G. versiforme* on the growth of wheat and lentil were evaluated in autoclaved soil under growth chamber conditions. *Glomus geosporum* increased the growth and yield of wheat, whereas *Glomus mosseae* only affected the shoot morphology compared to the uninoculated control. Similarly, lentil responded more to inoculation with *G. geosporum* than the other VAM. Inoculation of lentil with *G. geosporum* increased grain yield by 56% while *G. mosseae* and *G. versiforme* increased yield by 25 to 30%. The relative effectiveness of the three VAM on lentil (i.e., *G. geosporum* > *G. versiforme* > *G. mosseae*) was dependent on the extent of their colonization. Increased infection by the three VAM was related directly to P content of lentil shoot and grain, and also P uptake.

Improved P nutrition of a host plant is one key mechanism by which VAM influence crop growth (Mosse 1973a). In my study there was no consistent increase in P content nor an increase in shoot and grain yield of wheat plants inoculated with *G. mosseae*. However, there was a very striking effect of *G. mosseae* on the height of the wheat plants, which were taller than those inoculated with *G. geosporum*, and the uninoculated control plants. The cause of such an effect may not be easy to explain based on the
parameters measured in this study. It is possible that infection with *G. mosseae* affected the hormonal balance of wheat plants resulting in increased plant height. This should be evaluated in future studies.

The differences in the efficacy of *G. geosporum* and *G. mosseae* for wheat and lentil indicated some degree of host specificity in terms of their relative benefits to the host plant. This specificity was also reflected in the number of VAM spores produced in the soils cropped with these hosts. *Glomus geosporum* produced twice as many spores as *G. mosseae* regardless of the host. Furthermore, each of the VAM isolates produced three to four fold more spores in the lentil than in wheat soils.

The presence of both *G. geosporum* and *G. mosseae* in the growth substrate led to competition between the endophytes for the host. The more beneficial *G. geosporum* was for a host, the greater the negative impact of introducing *G. mosseae*. For example, *G. mosseae* reduced the efficacy of *G. geosporum* by 16% when co-inoculated on lentil, but had no effect when the host was wheat. Based on spore production, I also found that *G. geosporum* was more competitive than *G. mosseae* when co-inoculated to lentil and wheat. If a similar type of competition occurs under field conditions, there would be fewer *G. mosseae* spores in a continuous wheat or lentil field soil relative to the number of *G. geosporum* spores. Johnson et al. (1991a) found that the number of VAM spores of different species present at the beginning and at the completion of a five year crop rotation was affected by the cultivation of soybean and maize in monoculture, indicating that the host crop exerts an influence on the diversity of the VAM species in field soils. Furthermore, it may be that continuous growth of a single host over a long period may eliminate the VAM species which are not stimulated to sporulate by that specific host. The detection of *G. geosporum* and *G. mosseae* in the six soils used to isolate VAM species, and their presence at 10 study sites, indicated that these two VAM species were common in all the soils of Saskatchewan. Future studies on the population dynamics of
these two species in soils under different cropping practices might lead to some interesting conclusions about VAM competition.

Three VAM species, *G. geosporum*, *G. mosseae* and *G. versiforme*, which co-existed in Saskatchewan field soils, differed in their competitiveness and contribution to the growth of lentil and wheat. *Glomus geosporum* was a very competitive and a highly effective VAM for lentil and wheat. The sporulation ability of *G. geosporum* is yet another attribute which makes it a suitable candidate as a VAM inoculum. However, assessment of the competitive ability of *G. geosporum* based on sporulation under growth chamber conditions may not reflect its ability in the field. Although in most studies the competition between VAM species is evaluated on the basis of spore production (Daft and Hogarth 1983; Koomen et al. 1987), this method has limitations (Hepper et al. 1986; Hepper et al. 1988). For example, a VAM species may be highly competitive but produces few spores. Therefore, new techniques are needed to evaluate the competitive ability of different VAM.

A method to evaluate the competitive ability of a VAM should be based on the determination of infection morphology and percentage of infected roots occupied by an individual VAM species. However, it is a very difficult task to distinguish infection morphology of different VAM (Abbott and Robson 1978, 1982b). Simon et al. (1992) recently reported the possibility of detecting and quantifying specific VAM in host roots based on specific DNA probes for individual VAM species. This method appears promising for evaluating competition between VAM species both under growth chamber and field conditions.

Differences in infection morphology caused by *G. versiforme* in four hosts [leek (*Allium porrum* L.), wheat, sunflower and alfalfa] have been reported (Lackie et al. 1987), but the relationship of this difference to growth response was not studied.
found, that in general, *G. geosporum* infection in wheat and lentil was characterized by the production of abundant arbuscules, although the level of arbuscular infection in wheat was lower than that of lentil. The level of arbuscule production by *G. mosseae* was lowest. Thus I believe that the higher growth response of lentil to the selected VAM under growth chamber conditions was due to functional differences in the infection morphology. In addition, I found that lentil roots collected in different fields also contained abundant arbuscules. Based on my experience, and observations of plants from growth chamber studies, these arbuscules in field roots were more similar to those formed by *G. geosporum* than those of *G. mosseae*. This leads me to conclude that *G. geosporum* was the dominant VAM in the field. The use of DNA probes for detection of specific VAM species in the host root may be useful to test this hypothesis.

Arbuscules are the only characteristic structures produced by every known VAM species. Since arbuscules are important in nutrient transfer between host roots and the fungus, the size, shapes and dynamics of arbuscule production by different VAM species may be associated with the differential response of host to different VAM species. This aspect should to be investigated in the future VAM research.

Based on my observations on infection morphology of both wheat and lentil plants from growth chamber and fields, I believe that the relative benefits of VAM to wheat and lentil were different, even under field conditions. Coarse-rooted plant species with limited root hairs, such as legumes, are generally highly mycorrhizae dependent compared to plant species with finer roots, such as wheat (Baylis 1972; Gerdemann 1975). Generally, nitrogen fixing legumes have a high demand for P and maintain a higher tissue P concentration compared to wheat plants. Therefore, abundant arbuscules in the interface between the cell wall and plasma membrane of legume may provide a larger surface area for transfer of large amounts of P absorbed by the external hyphae. There is a need to develop methods to determine the P absorption per unit area of roots infected by
arbuscules in crops with different degrees of mycorrhizal dependency. Such studies may provide evidence to relate the differences in growth response of different crops directly to functional differences in the host fungus interphase. An alternative hypothesis to explain the observed variation in infection morphology of field grown lentil and wheat is that each crop formed a symbiosis with different, specific VAM. Unfortunately, no attempt was made to isolate VAM species from lentil field soils in my study.

The effect of cultivation of non mycorrhizal crops such as canola, or use of summerfallow as a break in continuous wheat and barley production systems was studied at several field sites. The purpose of this study was to identify cultivation practices that might affect VAM infection, and the contribution by indigenous VAM to the growth of wheat and barley. I observed that crop rotations with summerfallow and canola influenced the VAM spore numbers in soil and VAM infection in wheat and barley grown on these fields. The number of VAM spores in soil and the VAM infection in wheat and barley fields were reduced after cultivation of canola or following a summerfallow break, compared to fields where wheat was cultivated in the previous season. However, it appears that the effect of a canola crop or a summerfallow break may not be long lasting. This was indicated by a relatively large increase in spore numbers in these soils at the harvest of wheat and barley grown on canola and summerfallow fields. Nevertheless, the effect of canola and summerfallow on infective VAM propagule density may be different than the effect on spore numbers. Additional research should address this question.

In recent years the frequency of canola in rotation has increased in many parts of the province. The repeated growth of a non-host crop in a field may reduce (i) infective VAM propagules, (ii) VAM infection and (iii) benefits of indigenous VAM to crops grown subsequently in these fields. Soil erosion also is a severe problem in some parts of the province and this may also cause a reduction in VAM propagule density. Other workers have observed a drastic reduction in VAM spore numbers in soils due to summerfallow
(Black and Tinker 1979) or soil erosion (Hall 1980). Furthermore, they observed a positive response of crops to VAM inoculation in these soils. Abbott and Robson (1991b) stated that with current existing knowledge it is possible to: (i) identify sites used for agriculture where the VAM symbiosis is operating suboptimally, (ii) quantify the benefits that would result from increasing the rate and extent of colonization of roots by effective fungi, and (iii) to identify the most cost-effective method for managing VAM either by choice of agricultural practice or by inoculation with selected fungi. It is important, therefore, to study the infective propagule density of indigenous VAM in Saskatchewan agricultural fields frequently cropped to canola and in those fields subjected to severe soil erosion. These fields may be potentially useful for studying the growth response of wheat and lentil to the VAM inoculants produced in my research. Results of my growth chamber and field studies indicate a great potential of VAM inoculation in the production of these two crops.
5 LITERATURE CITED


Kothari, S.K., Marschner, H. and Romheld, V. 1991. Effect of a vesicular-arbuscular mycorrhizal fungus and rhizosphere micro-organisms on manganese in the


Morton, J.B. 1985. Variation in mycorrhizal and spore morphology of *Glomus occultum* and *Glomus diaphanum* as influenced by plant host and soil environment. Mycologia 77 : 192-204.


Walley, F. 1989. (University of Saskatchewan). Personal communication.


