Université de Montréal

Interactions mycorhizosphériques entre un champignon endomycorhizien arbusculaire (*Glomus intraradices*) et différents micro-organismes édaphiques

par

Martin Filion

Département de sciences biologiques

Faculté des arts et des sciences

Mémoire présenté à la Faculté des études supérieures

en vue de l'obtention du grade de

Maître ès sciences (M. Sc.)

en sciences biologiques

juin 1998

Université de Montréal

Faculté des études supérieures

Ce mémoire intitulé:

Interactions mycorhizosphériques entre un champignon endomycorhizien arbusculaire (*Glomus intraradices*) et différents micro-organismes édaphiques

présenté par:

Martin Filion

a été évalué par un jury composé des personnes suivantes :

Président du jury	Dr. Anne Bruneau
Directeur de recherche	Dr. J. André Fortin
Codirecteur	Dr. Marc St-Arnaud
Membre du jury	Dr. Chantal Hamel

Mémoire accepté le : 25.08.1998

SOMMAIRE

Les champignons endomycorhiziens arbusculaires (MA) peuvent réduire l'incidence et l'importance des maladies racinaires causées par des parasites du sol. Les mécanismes impliqués ne sont pas très bien caractérisés. Il a été suggéré que la réduction de susceptibilité aux maladies observée chez des plantes endomycorhizées puisse être dû à la présence d'interactions directes ou indirectes entre champignons MA et parasites du sol. Plusieurs modifications physiques, biochimiques et biologiques se produisent généralement dans la mycorhizosphère lorsqu'une plante acquiert un statut mycorhizien. Cette zone d'intense activité est alors sous le contrôle des exsudats racinaires ainsi que sous l'influence directe du ou des champignons MA présent(s). Il est démontré que la microflore du sol est qualitativement et quantitativement modifiée dans la mycorhizosphère comparativement à la rhizosphère d'une plante non-mycorhizée.

L'objectif de cette étude était d'améliorer notre compréhension des interactions de la mycorhizosphère afin d'expliquer la réduction des phénomènes de pathogénèse chez des plantes endomycorhizées. Deux axes principaux furent définis: 1) isoler des substances libérées par la phase extramatricielle d'un champignon MA et vérifier l'effet de ces substances sur la croissance de différents micro-organismes du sol; 2) suivre des populations de micro-organismes en sol à l'aide d'outils classiques et moléculaires afin d'en comprendre la dynamique mycorhizosphérique. Ces deux axes ont permis de déterminer si des interactions directes et indirectes entre champignons MA et parasites se produisent et comment cette dynamique s'opère.

Dans un premier temps, il fut démontré qu'un champignon MA, le *Glomus intraradices*, libère des substances via sa phase extramatricielle et que ces substances ont des effets très distincts au niveau de la croissance de différents micro-organismes. Ces effets vont d'une forte stimulation de croissance (*Trichoderma harzianum*, *Pseudomonas chlororaphis*) à une réduction (*Fusarium oxysporum*) en passant par l'absence d'effet significatif (*Clavibacter michiganensis*). Ainsi, il fut établit que le *G. intraradices* possède la capacité d'interagir directement avec des micro-organismes du sol.

Dans un deuxième temps, il fut démontré que le *G. intraradices* possède la capacité de réduire la population du *Clavibacter michiganensis* subsp. *michiganensis* dans la mycorhizosphère de tomates (*Lycopersicon esculentum*). La dynamique de population du *C. m. michiganensis* fut suivie en sol pendant 60 heures à l'aide d'un anticorps polyclonal anti-*C. m. michiganensis* couplé à l'utilisation de la technologie du DAS-ELISA. Cette étude a démontré que malgré l'inefficacité du *G. intraradices* à interagir directement avec le *C. m. michiganensis*, ce champignon MA a néanmoins la capacité d'interagir indirectement avec ce parasite racinaire. Les résultats suggèrent que cette interaction indirecte est engendrée par une modification des exsudats racinaires de plantes mycorhizées par comparaison à des plantes non-mycorhizées. De plus, des modifications mycorhizosphériques de populations bactériennes d'importance écologique furent démontrées.

TABLE DES MATIÈRES

SOMMAIRE iii

TABLE DES MATIÈRES v

LISTE DES TABLEAUX viii

LISTE DES FIGURES ix

LISTE DES ABRÉVIATIONS xi

REMERCIEMENTS xii

1. INTRODUCTION GÉNÉRALE 1

1.1 Systématique des champignons endomycorhiziens arbusculaires 1

1.2 Importance des symbioses endomycorhiziennes 2

1.3 Anatomie et physiologie 3

1.4 Bénéfices des symbioses endomycorhiziennes arbusculaires 4

1.5 Symbioses mycorhiziennes et sensibilité aux maladies racinaires 5

1.6 Objectifs visés 8

2. DIRECT INTERACTION BETWEEN THE AM FUNGUS *GLOMUS INTRARADICES* AND DIFFERENT RHIZOSPHERE MICROORGANISMS 9

RÉSUMÉ 10

SUMMARY 12

INTRODUCTION 14

MATERIALS AND METHODS 17

Ri T-DNA-transformed carrot root culture 17

Fungal and bacterial cultures 17

Description of the experimental units 17

Extraction and concentration of soluble substances released by the AM fungus 18

Effect of the extract on conidial germination of fungi or growth of bacteria 19

pH interaction 20

Statistical analyses 20

RESULTS 22

DISCUSSION 24

ACKNOWLEDGEMENTS 29

REFERENCES 30

TABLES 38

FIGURES 41

3. BIOLOGICAL CONTROL OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* POPULATIONS BY *GLOMUS INTRARADICES* 47

RÉSUMÉ 48

SUMMARY 49

INTRODUCTION 50

MATERIALS AND METHODS 53

Experimental systems 53

Soil and mycorrhizal inoculation 54

Plants, growth conditions and bacteria inoculation 54

Shoot dry weight, soil water content and arbuscular colonization 55

Root length, hyphal presence and soil pH 56

Bacterial extraction from soil 56

Bacterial culture, specificity tests and standard curve 57

DAS-ELISA protocol 58

Estimation of bacterial population 59

Statistical analyses 59

RESULTS 61

DISCUSSION 64

ACKNOWLEDGEMENTS 67

REFERENCES 68

TABLES 76

FIGURES 78

4. DISCUSSION GÉNÉRALE 86

4.1 Effets des substances libérées par le *Glomus intraradices* 86

4.2 Développement d'outils quantitatifs afin d'investiguer la mycorhizosphère .. 88

4.3 Interactions entre le *Glomus intraradices* et le *C. m. michiganensis* en sol 91

4.4 Conclusion 92

5. BIBLIOGRAPHIE GÉNÉRALE 94

LISTE DES TABLEAUX

CHAPITRE 2

Table 1 Effect of *Glomus intraradices* extraradical mycelium and concentration on the pH of a crude extract of the growth medium **38**

Table 2 Effect of the pH extract on conidial germination of *Fusarium oxysporum* f. sp. *chrysanthemi* and *Trichoderma harzianum* **39**

Table 3 Effect of the pH extract on growth of *Pseudomonas chlororaphis* and *Clavibacter michiganensis* subsp. *michiganensis* **40**

CHAPITRE 3

Table 1 Effect of mycorrhizal treatments on % of root length colonized by *Glomus intraradices* (AM) and shoot dry weight **76**

Table 2 Effect of mycorrhizal and compartmental treatments on hyphal presence, root length and soil pH **77**

LISTE DES FIGURES

CHAPITRE 2

Figure 1 Effect of a crude extract of the growth medium of *Glomus intraradices* on conidial germination (%) of *Fusarium oxysporum* f. sp. *chrysanthemi* **43**

Figure 2 Effect of a crude extract of the growth medium of *Glomus intraradices* on conidial germination (%) of *Trichoderma harzianum* **44**

Figure 3 Effect of a crude extract of the growth medium of *Glomus intraradices* on bacterial growth of *Pseudomonas chlororaphis* **45**

Figure 4 Effect of a crude extract of the growth medium of *Glomus intraradices* on bacterial growth of *Clavibacter michiganensis* subsp. *michiganensis* **46**

CHAPITRE 3

Figure 1 Compartmentalized growth unit system with a 5 week old tomato plant **80**

Figure 2 Relationship between diluted suspensions of a pure culture of *Clavibacter michiganensis* subsp. *michiganensis* and ELISA absorbance **81**

Figure 3 Effect of compartmentation on *Clavibacter michiganensis* subsp. *michiganensis* ELISA absorbance **82**

Figure 4 Effect of compartmentation on colony forming units of total bacterial number plated on TSA **83**

CHAPITRE 3

Figure 5 Effect of compartmentation on colony forming units of Gram- bacterial number plated on TSA+0.02% crystal violet **84**

Figure 6 Effect of compartmentation on colony forming units of total fluorescent pseudomonads number plated on king B+NPCC **85**

LISTE DES ABRÉVIATIONS

AM	arbuscular mycorrhizal
fw	fresh weight
MA	mycorhizien arbusculaire
PAR	photosynthetically active radiation
v	volume
w	weight

REMERCIEMENTS

Je voudrais exprimer toute ma gratitude à l'endroit de mon directeur de recherche, Dr. J. André Fortin, afin de m'avoir permis de découvrir le monde fascinant des symbioses mycorhiziennes. En plus des multiples contacts et opportunités qu'il m'a permis de réaliser, il a toujours été d'un support et d'une stimulation très enrichissante. Sa générosité et son souci d'aider les étudiants à aller toujours plus loin, en leur fournissant les outils adéquats, en font de lui non seulement une sommité scientifique dans le domaine des mycorhizes mais également quelqu'un à l'écoute des vrais besoins. Mon codirecteur, Dr. Marc St-Arnaud, fut également une autre personne ressource très importante au cours de mon cheminement. En plus de ses multiples compétences scientifiques et de sa rigueur à toute épreuve, il m'a beaucoup inspiré par son imagination et surtout son approche généreuse, calme et humaine.

Plusieurs collègues méritent également une admirable reconnaissance pour avoir facilité mon passage à l'I.R.B.V.. Je pense particulièrement à Stéphane Daigle pour ses précieux conseils sur la planification expérimentale et l'approche statistique juste à adopter au niveau des protocoles expérimentaux; Brigitte Vimard pour ses conseils, sa disponibilité et sa constante bonne humeur; mon grand ami Denis Lauzer pour son sens de l'humour et son expertise en culture *in vitro*; Claude Lafleur pour sa participation à la fabrication de différents systèmes expérimentaux; et à tout le personnel du secrétariat de l'I.R.B.V.

Je voudrais également remercier Dr. Yves Piché de l'Université Laval pour nous avoir fourni des racines de carottes transformées génétiquement ainsi que de l'inoculum

mycorhizien; Michel Lacroix du MAPAQ pour nous avoir fait parvenir différentes souches bactériennes; enfin, la compagnie Premier Tech pour nous avoir permis d'utiliser une de leurs souches bactériennes et également pour leur support constant dans le domaine des mycorhizes.

Un grand merci à tous ces gens, étudiants et personnel de recherche, qui ont participé à créer un environnement de travail agréable et permis le dévelopement de complicités et d'amitiés. La liste serait trop longue, mais je pense à vous tous. Enfin, un dernier gros merci va à mes parents pour leur support et leur compréhension lors de mon cheminement.

1. INTRODUCTION GÉNÉRALE

1.1 Systématique des champignons endomycorhiziens arbusculaires

La systématique des champignons endomycorhiziens arbusculaires (MA) fut maintes fois remaniée au cours des dernières décennies (Gerdemann & Trappe 1974; Gerdemann & Trappe 1975; Morton 1988; Morton & Benny 1990; Trappe & Schenck 1982). Ces modifications arrivèrent de concert avec l'explosion récente de l'intérêt qu'a suscité le domaine des symbioses mycorhiziennes. L'arrivée de nouvelles techniques et outils modernes dans ce champ d'intérêt a permis des progrès majeurs. Ainsi, les précieuses informations recueillies par la systématique classique se sont vues enrichir par des données supplémentaires provenant de la systématique contemporaine, notamment grâce à l'utilisation de techniques biochimiques (Bonfante-Fasolo *et al.* 1986), sérologiques (Aldwell *et al.* 1985) et électrophorétiques (Hepper *et al.* 1988). La classification actuelle regroupe les champignons endomycorhiziens arbusculaires dans le règne des Eumycota, l'embranchemant des Zygomycota, la classe des Zygomycètes et l'ordre des Glomales (Morton & Benny 1990). Deux sous-ordres, Glomineae et Gigasporineae, furent créés afin de différencier respectivement les espèces produisant des vésicules et d'autres n'en produisant pas. En moins de 50 ans, le nombre d'espèces répertorié est passé de 35 à plus de 150 (Dalpé 1995). Récemment, à l'aide de marqueurs moléculaires, l'origine des champignons MA fut située entre 462 et 353 millions d'années avant notre ère (Simon *et al.* 1993). Ces estimés font coïncider leur apparition avec celle des premières plantes terrestres. De plus, les informations obtenues à l'aide d'outils modernes furent corroborées par la découverte de fossiles de plantes du début du

Dévonien, révélant la présence de structures fongiques identiques aux arbuscules, structures caractéristiques d'une symbiose mycorhizienne fonctionnelle (Remy *et al.* 1994; Taylor *et al.* 1995).

1.2 Importance des symbioses endomycorhiziennes

Grâce à leur origine très ancienne, ces symbiotes obligatoires ont su développer des associations avec la grande majorité des plantes de la flore contemporaine. Ils forment des associations symbiotiques avec tous les grands groupes de plantes vasculaires présentant des racines, incluant les Angiospermes, les Gymnospermes et les Ptéridophytes (Smith & Read 1997). De plus, ils ont été observés chez des plantes sans racines vraies, telles des gamétophytes de Bryophytes et de Psilophytes (Peterson *et al.* 1981; Pocock & Duckett 1984; Pocock & Duckett 1985). On ne connaît pas jusqu'à maintenant de véritables spécificités d'associations entre les champignons MA et les plantes (Harley 1985). Ainsi, un même champignon MA peut former une association symbiotique avec diverses espèces de plantes. De plus, une même plante peut être mycorhizée par différentes espèces de champignons MA. Néanmoins, certaines préférences végétales semblent exister pour certaines souches endomycorhiziennes (Vasanthakrishna & Bagyaraj 1993) et la dépendance mycorhizienne peut varier entre cultivars distincts d'une même espèce végétale (Hetrick *et al.* 1993). Aujourd'hui, environ 95% des plantes vasculaires appartiennent à des familles mycorhiziennes (Trappe 1987), conférant à cette association symbiotique une importance fondamentale dans la vaste majorité des écosystèmes terrestres répartis sur tous les continents.

1.3 Anatomie et physiologie

La symbiose mycorhizienne est initiée directement par un tube germinatif provenant de propagules du sol, telles des spores, des hyphes ou des sections de racines déjà colonisées. La formation d'un appressorium précède la pénétration d'une racine, suivie par une ramification intercellulaire à l'intérieur de la région corticale. La croissance majoritairement intercellulaire soutient la formation d'arbuscules intracellulaires, véritables interfaces d'échanges entre la plante et le champignon (Bonfante & Perotto 1995). Les espèces appartenant aux Glomineae forment également des renflements terminaux d'hyphes inter- ou intracellulaires, appelés vésicules, qui constituent des sites d'entreposage de lipides et représentent également une source de propagules. Environ 80% des champignons MA forment des vésicules (Smith & Read 1997). Le mycélium intraracinaire est liée, à l'extérieur des racines, à une croissance d'hyphes qui s'étend dans le sol. Ce réseau d'hyphes extracellulaires forme ce que l'on nomme la phase extraracinaire ou extramatricielle. Le volume de sol sous l'influence de cette phase, nommé mycosphère, est considérable et généralement de beaucoup supérieur à la surface explorée par les racines, ou rhizosphère. L'ensemble de la surface du sol sous l'influence des racines et des hyphes se nomme ainsi mycorhizosphère (Linderman 1988). Après un certain temps, des spores se développent au niveau de la phase extramatricielle et sont aptes, lorsqu'en conditions favorables, à germer et coloniser de nouvelles plantes. La présence de sporocarpes peut être observée chez certaines espèces. Enfin, mentionnons qu'aucune démonstration de sexualité n'a été faite jusqu'à ce jour chez les Glomales. Il apparaît que ces organismes asexués assurent une variabilité génétique par mutations et anastomoses d'hyphes aseptées, permettant ainsi

des échanges de noyaux entre isolats d'une même espèce et entre espèces différentes (Smith & Read 1997).

1.4 Bénéfices des symbioses endomycorhiziennes arbusculaires

L'association symbiotique mycorhizienne est généralement de type mutualiste. Le champignon acquiert des nutriments de la plante afin de survivre puisqu'il est un symbiote obligatoire. Il reçoit principalement des sucres qui sont transloqués via la racine et acheminés au champignon par les arbuscules. En retour, la plante bénéficie d'une importante exploration du sol par la phase extramatricielle du champignon et acquiert ainsi plus de nutriments, particulièrement des ions peu mobiles comme le zinc, le cuivre et surtout le phosphore (Smith & Gianinazzi-Pearson 1988). De plus, l'approvisionnement en eau semble amélioré chez les plantes bénéficiant d'un statut mycorhizien. Ces dernières résistent mieux aux stress hydriques (Davies *et al.* 1992; Nelsen & Safir 1982; Subramanian *et al.* 1995) et à la salinité (Allen & Cunningham 1983). La phase extramatricielle libère dans le sol des protéines qui exercent un impact positif au niveau de l'aggrégation, améliorant ainsi la stabilité du substrat (Sutton & Sheppard 1976; Wright *et al.* 1996; Wright & Upadhyaya 1996). Enfin, les plantes endomycorhizées jouissent d'une protection accrue contre les maladies causées par des parasites racinaires (Azcón-Aguilar & Barea 1996; Bagyaraj 1984; Caron 1989; Dehne 1982; Hooker *et al.* 1994; St-Arnaud *et al.* 1995).

1.5 Symbioses mycorhiziennes et sensibilité aux maladies racinaires

Malgré la réduction de la susceptibilité des plantes endomycorhizées aux maladies racinaires, les mécanismes impliqués ne sont pas bien connus. Plusieurs hypothèses ont néanmoins été proposées afin de tenter d'expliquer ce phénomène: 1)la stimulation des mécanismes de défense naturels de la plante, 2) une amélioration du statut nutritionnel de la plante, 3) des interactions directes entre le champignon MA et des parasites, 4) des interactions indirectes conduisant à une modification importante de la microflore du sol (St-Arnaud *et al.* 1995). Il est très vraisemblable que plus d'un mécanisme intervient en réponse à différents parasites.

1.5.1 Stimulation des mécanismes de défense naturels de la plante

Plusieurs études ont démontré que la colonisation d'une racine par un champignon MA induit faiblement et de façon transitoire l'activation de diverses voies métaboliques reliées aux mécanismes de défense naturels de la plante. Cette stimulation est rapidement suivie par une suppression qui rétablit les niveaux de substances induites à ceux des témoins (Harrison & Dixon 1993; Lambais & Mehdy 1993; Spanu *et al.* 1989; Spanu & Bonfante-Fasolo 1988; Vierheilig *et al.* 1994; Volpin *et al.* 1994). Cette réponse a souvent été interprétée comme une élicitation, potentiellement réactivée plus rapidement lorsque la plante entre en contact avec un parasite. Cependant, le niveau de stimulation demeure toujours de beaucoup inférieur à celui d'une stimulation de ces mêmes mécanismes par un parasite. Ainsi, il n'y a présentement aucune certitude à l'effet que l'activation de ces mécanismes par un champignon MA soit impliquée dans la

réduction de la susceptibilité aux maladies observée chez les plantes endomycorhizées (Gianinazzi-Pearson *et al.* 1996; Smith & Read 1997). Il n'existe actuellement qu'une seule étude démontrant clairement l'activation des mécanismes de défense d'une plante endomycorhizée lorsque confrontée à un parasite (Benhamou *et al.* 1994). Toutefois, cette étude a été effectuée en conditions très artificielles, sur des racines de carottes transformées génétiquement, qui sont normalement résistantes au pathogène employé dans l'étude.

1.5.2 Amélioration du statut nutritionnel de la plante

Une amélioration du statut nutritionnel de la plante augmente généralement la résistance aux stress biotiques et abiotiques (Agrios 1997). Il a souvent été proposé que les plantes mycorhiziennes puissent mieux résister aux attaques de parasites grâce à une meilleure accessibilité aux nutriments du sol, particulièrement en ce qui à trait à la nutrition phosphatée. Le fait que les plantes mycorhizées aient accès à plus de nutriments peut certainement compenser une diminution de biomasse causée par l'attaque de portions racinaires par des parasites. Néanmoins, il a été clairement démontré par plusieurs études que l'amélioration du statut nutritionnel n'est pas en cause afin d'expliquer une augmentation de résistance face à divers parasites (Caron *et al.* 1986; Newsham *et al.* 1995; St-Arnaud *et al.* 1994).

1.5.3 Interactions directes et indirectes entre champignons MA et parasites

Il est difficile de clairement distinguer entre des interactions directes et indirectes qu'entretiennent un champignon MA et différents parasites. La majorité des études traitant de ces aspects furent effectuées à l'aide de cultures en pots, en présence d'une plante, d'un ou plusieurs champignons MA et d'un ou plusieurs parasites, pour une condition édaphique donnée (Caron *et al.* 1986; Catska 1994; Garcia-Garrido & Ocampo 1988; Garcia-Garrido & Ocampo 1989; Hwang *et al.* 1992; Kaye *et al.* 1983; McAllister *et al.* 1994; St-Arnaud *et al.* 1994). Ces études ont souvent démontré des effets très nets de champignons MA sur la réduction de symptômes de maladies ou directement de populations de parasites en sol. Néanmoins, il est souvent impossible de départager l'effet direct de substances libérées dans l'environnement par un champignon MA sur un parasite donné de l'impact que ces substances peuvent avoir sur différents micro-organismes, qui, à leur tour, peuvent interagir avec des parasites. De plus, l'effet indirect peut également s'opérer via une modification d'exsudation racinaire causée par un statut mycorhizien. Il est clairement démontré que les exsudats racinaires de plantes endomycorhizées sont différents de ceux provenant de plantes non-mycorhizées (Bansal & Mukerji 1994; Schwab *et al.* 1984).

Toutes ces modifications contribuent à créer une modification qualitative, quantitative et spatiale des populations microbiennes de la mycorhizosphère (Andrade *et al.* 1997; Catska 1994; Citernesi *et al.* 1996; Meyer & Linderman 1986; Paulitz & Linderman 1989; Posta *et al.* 1994). Deux zones distinctes d'interactions peuvent être définies: 1) le rhizoplan et le sol adjacent de la rhizosphère et 2) la mycosphère. La mycosphère, sous l'influence des hyphes du champignon MA, subit des modifications

chimiques et de pH (Bago & Azcón-Aguilar 1997). Il fut également démontré que des substances, notamment des glycoprotéines, sont libérées par la phase extramatricielle de différents champignons MA (Wright *et al.* 1996; Wright & Upadhyaya 1996).

1.6 Objectifs visés

Le présent projet vise à mieux comprendre les mécanismes impliqués dans la réduction des phénomènes de pathogénèse végétale chez des plantes endomycorhizées. Parmi tous les mécanismes proposés par le passé, certains semblent avoir plus d'impact que d'autres. L'existence d'interactions directes ou indirectes entre champignons MA et parasites du sol dans la mycorhizosphère, afin d'expliquer la réduction de suceptibilité aux maladies observée chez des plantes endomycorhizées, semble ainsi constituer un axe majeur de ce champs d'intérêt.

Le premier chapitre de ce mémoire décrit la mise en évidence de l'effet de substances libérées par la phase extramatricielle d'un champignon MA (*Glomus intraradices*) sur la croissance de différents micro-organismes du sol (*Fusarium oxysporum*, *Trichoderma harzianum*, *Pseudomonas chlororaphis* et *Clavibacter michiganensis*). Le second chapitre traite de la dynamique de populations de micro-organismes en sol, sous l'influence du *Glomus intraradices*, notamment du *Clavibacter michiganensis* subsp. *michiganensis*, à l'aide d'outils classiques et moléculaires afin d'en comprendre la dynamique mycorhizosphérique. Les deux chapitres de ce mémoire sont présentés sous forme de manuscrit et sont rédigés en anglais. Le premier manuscrit est soumis pour publication à la revue The New Phytologist et le second manuscrit sera soumis à la revue Phytopathology.

2. DIRECT INTERACTION BETWEEN THE AM FUNGUS *GLOMUS INTRARADICES* AND DIFFERENT RHIZOSPHERE MICROORGANISMS

Ce manuscrit, ayant comme coauteurs: M. Filion, M. St-Arnaud and J. A. Fortin, est soumis à la revue The New Phytologist. Ce travail traite de l'isolation de substances libérées par la phase extramatricielle du *Glomus intraradices* et de l'effet de ces substances sur la croissance de différents micro-organismes représentatifs de la mycorhizosphère. Cette étude effectuée en conditions *in vitro* apporte une démonstration claire d'un mécanisme physiologique fondamental des champignons MA. Ce travail s'inscrit comme assise en vue d'études à effectuer en conditions plus complexes.

RÉSUMÉ

Les champignons endomycorhiziens arbusculaires (MA) peuvent réduire l'incidence et l'importance des maladies racinaires causées par des parasites. Les mécanismes impliqués ne sont pas bien caractérisés. Nous avons utilisé un système expérimental *in vitro* afin de vérifier l'hypothèse selon laquelle la phase extraracinaire de champignons MA peut interagir directement avec des micro-organismes de la mycosphère et directement ou indirectement reduire des populations de parasites du sol. Le système utilisé a permis l'isolation de substances solubles libérées par la phase extraracinaire du *Glomus intraradices*. Le champignon MA fut cultivé sur racine de *Daucus carota* transformées dans un compartiment tandis que seulement la phase extraracinaire du champignon MA fut en mesure de croître dans le deuxième compartiment d'un vase de Pétri à deux compartiments. Une technique de congélation et centrifugation fut développée pour l'extraction et la concentration des substances présentes dans le compartiment ne contenant que le champignon MA. Quatre micro-organismes représentatifs de la rhizosphère furent sélectionnés et la germination conidienne (champignons) ou la croissance bactérienne (bactéries) de ces micro-organismes furent étudiées en présence ou absence (témoins) des extraits de substances isolées. Par comparaison aux témoins, les résultats indiquèrent que la croissance du *Pseudomonas chlororaphis* et la germination conidienne du *Trichoderma harzianum* furent stimulées en présence des extraits provenant du compartiment contenant le champignon MA. De plus, la germination conidienne du *Fusarium oxysporum* f. sp. *chrysanthemi* fut réduite tandis que la croissance du *Clavibacter michiganensis* subsp. *michiganensis* ne fut pas affectée par ces mêmes substances. Les effets mesurés furent

généralement directement corrélés avec la concentration des extraits. Des différences de pH furent notées entre les extraits contenant des substances libérées par le champignon MA par rapport aux témoins mais aucune influence significative du pH ne fut notée au niveau de la germination conidienne ou de la croissance bactérienne. Ceci confirme que les substances libérées dans le milieu de culture par le champignon MA constituent la meilleure hypothèse pouvant expliquer la croissance différentielle des micro-organismes étudiés. Les résultats suggèrent que des interactions directes existent entre champignons MA et micro-organismes du sol, ce qui peut conduire à une modification de l'équilibre microbien nuisible à la croissance de parasites racinaires.

Mots-clés : champignon MA, *Glomus intraradices*, phase extraracinaire, parasites, interactions.

SUMMARY

AM fungi can reduce the incidence and importance of plant root diseases caused by pathogens. The mechanisms involved are not well characterized. We used an *in vitro* experimental system to test the hypothesis that the extraradical mycelium of AM fungi can interact directly with the microorganisms of the mycosphere and directly or indirectly reduce the population of plant pathogens. This system permitted the isolation of soluble substances released by the extraradical mycelium of *Glomus intraradices*. The AM fungus was grown on *Daucus carota* transformed roots in one compartment of two-compartment Petri dishes while only the extraradical mycelium of the AM fungus was allowed to grow in the second compartment. A freezing and centrifugation technique was developed for the extraction and concentration of substances present in the compartment containing only the AM fungal mycelium. Four soil inhabiting microorganisms were selected and conidial germination (fungi) or growth (bacteria) of these microorganisms was studied in the presence and absence (control) of the extract. In comparison with the control, the results indicated that both the growth of *Pseudomonas chlororaphis* and the conidial germination of *Trichoderma harzianum* were stimulated in the presence of the AM fungal extract. In contrast, conidial germination of *Fusarium oxysporum* f. sp. *chrysanthemi* was reduced while the growth of *Clavibacter michiganensis* subsp. *michiganensis* was not affected. In general, the measured effects were directly correlated with extract concentration. Differences in pH were noted between the extract containing substances released by the AM fungus and the non-AM control, but no significant influence of the pH on growth or conidial germination was observed, confirming that substances released by the AM fungus in the

growth medium was the main factor explaining differential growth of the microorganisms tested. The results suggest that direct interactions between AM fungi and soil microorganisms may lead to changes in microbial equilibrium detrimental to pathogens.

Key words : AM fungi, *Glomus intraradices*, extraradical mycelium, pathogens, interactions.

INTRODUCTION

An intimate relationship between arbuscular mycorrhizal (AM) fungi and plants has existed for at least 350 millions years (Remy *et al.* 1994; Simon *et al.* 1993; Taylor *et al.* 1995). This coevolution has involved numerous interactions at the ecological, physiological and molecular levels between these organisms during the long development of the symbiosis. Today, about 95 % of the world's existant species of vascular plants belong to families that are typically mycorrhizal, making this association of fundamental importance in all ecosystems (Trappe 1987).

In addition to the well known beneficial nutritional effect of the symbiosis on plants (Smith & Gianinazzi-Pearson 1988) and the positive impact on hydric status (Davies *et al.* 1992; Nelsen & Safir 1982) and soil aggregation (Sutton & Sheppard 1976; Tisdall 1994), AM fungi can reduce the incidence and importance of root diseases (Azcón-Aguilar & Barea 1996; Bagyaraj 1984; Caron 1989; Dehne 1982; Hooker *et al.* 1994; St-Arnaud *et al.* 1995a;).

Stimulation of host plant disease resistance mechanisms, improvement of plant nutrition, direct interaction between AM fungal mycelium and pathogens, or indirect effects through changes in soil microflora, have all been proposed to explain the influence of AM fungi on disease development (St-Arnaud *et al.* 1995a). Many studies have shown that relative to non-mycorrhizal controls, AM fungal colonization of host plants may induce a slight and transient activation of metabolical pathways related to disease resistance mechanisms, which are followed by suppression of the same mechanisms (Harrison & Dixon 1993; Lambais & Mehdy 1993; Spanu & Bonfante-Fasolo 1988; Spanu *et al.* 1989; Vierheilig *et al.* 1994; Volpin *et al.* 1994). This

response has often been interpreted as an elicitation process, potentially capable of being more rapidly reactivated if a plant becomes challenged by a pathogen. However, the magnitude of the plant reaction is always low compared to pathogenic interactions and there is still no clear evidence that the mechanisms activated by colonization with AM fungi are directly involved in the reduction of plant root diseases in mycorrhizal plants (Gianinazzi-Pearson *et al.* 1996; Smith & Read 1997). Activation of plant defense mechanisms in mycorrhizal roots challenged by a pathogen has been shown by Benhamou *et al.* (1994), but their study was done under artificial conditions on transformed carrot roots (*Daucus carota* L.), which are normally resistant to the pathogen they examined. It therefore needs confirmation in a non-genetically transformed entire plant species system to validate the contribution of this type of mechanisms in agrosystems or in natural ecosystems.

An improvement of plant nutrition often confers more resistance to abiotic or biotic stresses (Agrios 1997). In some situations, the greater availability of nutrients to mycorrhizal plants can compensate for attack and decay of root sections by pathogens and cause damages to be expressed to a lesser extent (Dehne 1982; Smith & Gianinazzi-Pearson 1988). However, this does not signify that the improvement of plant nutrition resulting from a mycorrhizal status, especially in phosphorus, always increases or is the direct cause of resistance to root pathogens. It has been shown that a better phosphorus nutrition of mycorrhizal plants is not involved in reduction of disease symptoms or pathogen populations in different plant-pathogen systems (Caron *et al.* 1986; Newsham *et al.* 1995; St-Arnaud *et al.* 1994; St-Arnaud *et al.* 1997).

Qualitative, quantitative and spatial shifts in the population composition of mycorrhizosphere microorganisms induced by AM fungal colonization are now well

demonstrated (Andrade *et al.* 1997; Linderman 1988; Paulitz & Linderman 1989; Linderman & Paulitz 1990; Paulitz & Linderman 1991; Posta *et al.* 1994). Two zones of interactions can be defined : 1) the rhizoplan and the surrounding rhizosphere soil, and 2) the mycosphere. The root exudation is modified both qualitatively and quantitatively by the presence of an active AM symbiosis (Bansal & Mukerji 1994; Schwab *et al.* 1984). The extraradical mycelium of AM fungi also influences the chemical composition and pH of the soil (Bago & Azcón-Aguilar 1997). Extraradical mycelium of AM fungi is known to release substances into the soil (Wright *et al.* 1996; Wright & Upadhyaya 1996). However, we know of no empirical studies confirming that a release of substances into the mycorrhizosphere by an AM fungal mycelium can significantly shift microbial populations in the soil.

St-Arnaud *et al.* (1996) designed a two-compartment *in vitro* experimental system in which the AM fungal mycelium is separated from the host roots. This system allows the isolation of soluble substances released by the extraradical mycelium of an AM fungus grown under monoxenic conditions. This approach was used here to demonstrate the release of substances in the fungus only compartment and to determine the effect of the crude extract on various bacteria and fungi. The purpose of our study was to test the effect of a crude extract obtained from the growth medium of *Glomus intraradices* Schenck & Smith on conidial germination of two fungi, *Fusarium oxysporum* Schl. f. sp. *chrysanthemi* G.M. & J.K. Armstrong & Littrell and *Trichoderma harzianum* Rifai, and on the growth of two common soil bacteria species, *Pseudomonas chlororaphis* (Guignard & Sauvageau) Bergey *et al.* and *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis *et al.*

MATERIALS AND METHODS

Ri T-DNA-transformed carrot root culture

Ri T-DNA-transformed carrot roots (*D. carota*) were grown on a minimal (M) medium previously described by Bécard & Fortin (1988) but solidified with 0·4 % (w/v) gellan gum (ICN Biochemical, Cleveland, Ohio) instead of 1 % (w/v) bacto-agar (modified M medium). The carrot roots were colonized with the AM fungus *Glomus intraradices*. The spore extraction technique and mycorrhizal colonization establishment is described in St-Arnaud *et al.* (1996).

Fungal and bacterial cultures

The root pathogen *F. o. chrysanthemi* (ATCC 66279) and a non-pathogenic strain of *T. harzianum* (ATCC 52443) were grown on potato-dextrose agar (Difco) in darkness at 24°C. *P. chlororaphis* (Premier Tech 63-28) and *C. m. michiganensis* (MAPAQ 1059) were grown on King B agar in darkness at 28°C.

Description of the experimental units

Two-compartment Petri dishes (100 X 15mm) were used. One compartment was filled entirely with 25 ml of modified M medium. The other compartment received 8 ml of 4 % (w/v) gellan gum with 0·44 % (w/v) $MgCl_2 \cdot H_2O$, pH 5·5 (water Gel Gro). After setting of the water Gel Gro, another 2 ml of water Gel Gro was pipetted into the same

compartment. The Petri dishes were placed at an angle in order to form a slope with the dividing wall, of 0·5 to 1 mm from the top wall of the water Gel Gro medium. This slope was made in order to facilitate the passage of the AM fungus from one compartment to the other. Mycorrhizal *D. carota* transformed roots were transferred into the compartment containing modified M medium (proximal compartment). The dishes were incubated upside down at a 45° angle in the dark at 28°C until the extraradical mycelium had crossed the central wall and had grown into the distal compartment to cover approximately 50 % of all the area. The cultures were examined weekly and roots were trimmed aseptically as needed in order to prevent their growth in the distal compartment. Controls consisted of non-AM Ri T-DNA-transformed carrot roots cultured as described.

Extraction and concentration of soluble substances released by the AM fungus

Petri dishes were selected between 8-16 weeks after inoculation for the extraction process. Gelosis containing the AM mycelium (absent in the control) from the distal compartment of selected Petri dishes was removed and frozen for 5 h at -20°C, and then thawed at 22°C in order to liquefy the gelosis. The suspension obtained was centrifuged for 30 min at 39200 **g**. The pellet was discarded and the supernatant was frozen at -20°C for at least 12 h, freeze-dried for 20 h and kept frozen at -20°C until ready for use.

Effect of the extract on conidial germination of fungi or growth of bacteria

Freeze-dried extract was thawed at 22°C and dilutions were carried out in sterile distilled water. The extract was filtered through a 0·22 µm filter. Concentrations of 10X, 5X, 2·5X and 1X of the initial medium concentration were prepared. One hundred µl of each dilution were pipetted into eight wells of a 96 microwell plate (four dilutions from the AM fungus extract and four dilutions from the non-AM control). Fifty µl of conidial or bacterial suspensions of a selected microorganism were added to each of the eight wells. For bacteria, reading of the initial absorbance was taken for each of the eight wells of each plate after adding the 50 µl suspension of the designated microorganism. Seven or eight microwell plates were prepared for each repetition of the bioassay and one of these plates was used for determining the pH. The experiment was repeated 3 to 5 times for each microorganism.

Fusarium oxysporum f. sp. *chrysanthemi* and *T. harzianum* conidial suspensions were prepared in sterile distilled water and spore concentration was adjusted to $1{\cdot}0 \times 10^5$ conidia·ml^{-1} using an hematocytometer. Concentration of *P. chlororaphis and C. m. michiganensis* suspensions were adjusted by reading the absorbance (O.D.) using an Emax microplate reader (Molecular devices, Menlo Park, CA, USA). The density of the bacterial suspensions were adjusted to approximately 0·011 O.D. at 650 nm ($1{\cdot}5 \times 10^8$ bacteria ml^{-1} for *P. chlororaphis* and $3{\cdot}5 \times 10^7$ bacteria ml^{-1} for *C. m. michiganensis*). Concentrations were determined by comparison with a standard curve prepared from known concentrations plated on King B agar medium. The plates were incubated until germination tubes were evident on germinated conidia for fungi (*F. o. chrysanthemi*: 6 h at 22°C, *T. harzianum*: 14 h at 22°C) or until optimal growth for the bacteria (*P.*

chlororaphis: 24 h at 28°C, *C. m. michiganensis* 48 h at 28°C). The assessment of germination or growth for each microorganism was evaluated after incubation. For the fungi, conidial germination was determined on an inverse microscope at 320 X. The plates were first placed in an ice bath at 2°C to stop germination, and 100 conidia were randomly examined in each well for presence of germination tubes. For the bacteria, growth was assessed by the difference between final and initial O.D. values.

pH interaction

For each experiment, pH was measured using a microelectrode in each of the eight wells of one prepared microplate. To assess the possible role of the pH on the results, four wells of a microplate were filled with 100 µl of the water Gel Gro extract and the pH was adjusted to 6·5, 7·0, 7·5 and 8·0 with NaOH. Six plates were prepared for each microorganism tested and the experiment was repeated four times. Fifty µl of conidial or bacterial suspensions were prepared as described above and added to the four wells of each plate. The plates were incubated as previously described, and conidial germination or bacterial growth was determined.

Statistical analyses

The experimental design consisted of 35 blocks for *F. o. chrysanthemi*, 35 blocks for *T. harzianum*, 18 blocks for *P. chlororaphis* and 30 blocks for *C. m. michiganensis* (3-5 repetition of the experiment, each with 6 or 7 plates). In the statistical analysis model, one plate represented one block. Statistical analyses were done with Correlation

and General Linear Model procedures of the SAS statistical software (SAS Institute Inc. 1996). Conidial germination and bacterial growth were analysed by an ANOVA. A rank transformation (Lehmann, 1975) was performed for the *T. harzianum*, *C. m. michiganensis* and *P. chlororaphis* data in order to meet the requirements of the tests. *A posteriori* comparisons between treatments were done by Tukey's student range tests.

RESULTS

The conidial germination of *F. o. chrysanthemi* was significantly reduced ($P<0{\cdot}001$) after 6 h of incubation at 22°C for each concentration of AM fungus extract as compared with the non-AM control (Fig. 1). Increasing the concentration of the extract (for both the AM fungus extract and the non-AM control) also reduced the conidial germination of *F. o. chrysanthemi* ($P<0{\cdot}001$). A significant interaction was observed between AM inoculation and extract concentration treatments ($P<0{\cdot}001$).

The conidial germination of *T. harzianum* was significantly increased ($P<0{\cdot}001$) after 14 h of incubation at 22°C for each concentration of AM fungus extract as compared with the non-AM control (Fig. 2). The extract concentration (AM fungus extract and non-AM control) had a significant effect on *T. harzianum* conidial germination ($P<0{\cdot}001$). In the control extract, higher concentrations lowered the germination. In the presence of the AM fungus extract, germination was significantly higher only at the 2·5 X factor concentration. A significant ($P<0{\cdot}001$) interaction was observed between AM inoculation and extract concentration treatments.

Pseudomonas chlororaphis growth was significantly increased ($P<0{\cdot}001$) after 24 h of incubation at 22°C for each concentration of the AM fungus extract as compared with the non-AM control (Fig. 3). Increasing the extract concentration (AM fungus extract and non-AM control) increased the growth of *P. chlororaphis* ($P<0{\cdot}001$). A significant ($P<0{\cdot}001$) interaction was observed between AM inoculation and extract concentration treatments.

Clavibacter michiganensis subsp. *michiganensis* growth was not significantly affected ($P=0{\cdot}802$) after 48 h of incubation at 22°C by the presence of AM fungus

extract as compared to the non-AM control (Fig. 4). The extract concentration (AM fungus extract and non-AM control) had a significant effect on *C. m. michiganensis* growth ($P<0{\cdot}001$). A significant ($P<0{\cdot}05$) interaction was observed between AM inoculation and extract concentration treatments.

Differences of pH for the AM fungus extract and the non-AM control extract were noted at different concentration factors. A range of pH from approximately 6·5 to 7·5 was observed (Table 1). Values of pH between 6·5 and 8·0 had no significant effect on the conidial germination of *F. o. chrysanthemi* ($P=0{\cdot}604$) and *T. harzianum* ($P=0{\cdot}443$) (Table 2). However, there was a significant effect of pH on the growth of *P. chlororaphis* and *C. m. michiganensis* ($P<0{\cdot}001$) (Table 3). The growth of *P. chlororaphis* was significantly enhanced at pH values of 7·0 and 7·5 as compared with 6·5 and 8·0. For *C. m. michiganensis*, the growth was higher at pH values of 7·5 and 8·0 as compared to 6·5 and 7·0.

DISCUSSION

The results showed that a crude extract of the growth medium of *G. intraradices* have important effects on different soil microorganisms. The effect varied for each microorganism tested and was generally correlated with the extract concentration. To eliminate the effect of pH as a factor directly influencing growth of microorganisms, the effect of pH was examined. Our results further indicate that the pH of the growth medium variously affects different microorganisms. For the fungi, no significant pH effect was obtained, confirming that the pH modification induced by AM fungal colonization does not affect conidial germination. However, bacterial growth was significantly affected by the pH extract. For bacteria, lower pH levels near 6·5 decreased growth as compared with values of 7·0 for *P. chlororaphis* and 7·5 for *C. m. michiganensis*. However, even if AM colonization lowers the pH of the crude extract near 6·5, especially at the higher concentration factor, *P. chlororaphis* growth was still significantly higher than that observed on the control plates. This indicates that the decrease in pH of the growth medium induced by AM colonization is not a factor in the growth enhancement of *P. chlororaphis* observed in this experiment. Instead, we propose that release of substances into the growth medium by the AM fungal mycelium likely explain the observed effects on the growth of the microorganisms studied.

The system used to produce the crude extract and to test its effects on the growth of different microorganisms did not allow us to estimate the concentration of substances released near the extraradical mycelium of an AM fungus in natural conditions, which might be significantly higher than the 10 X concentration factor used in this experiment. In soil, concentrations are higher near the hyphae and gradually decrease with distance

from the mycelium, creating a relatively small zone of interaction. The fact that the experimental system used did not permit us to isolate this zone of higher concentration, may underestimate the magnitude of stimulation or inhibition between AM mycelium and soil microorganisms. It is also possible that AM fungi release non-soluble and/or volatile substances that can have an effect on soil microorganisms. Such substances were not recovered with this experimental system. Another limitation of the protocol is that it was not possible to distinguish between intra-hyphal and substances released by the mycelium into the environment. Nevertheless, in natural conditions, both kinds of substances would be present in the mycorrhizosphere due to frequent releases of hyphal material of AM extraradical structures into the soil, mainly generated by the frequent disruption of AM mycelium by different organisms and also by the death and natural decomposition of hyphae at frequent intervals (Fitter & Garbaye 1994; Klironomos & Ursic 1998; Larsen & Jakobsen 1996; Moore *et al.* 1985; Reddell *et al.* 1997; Thimm & Larink 1995).

Although release of substances from AM fungi are not well documented (Wright *et al.* 1996; Wright & Upadhyaya 1996), it is well known that root exudates modify strongly the microbial composition and activity in the rhizosphere. Different substances released by the roots, such as soluble sugars, phenolics, organic acids, amino acids, antibiotics and volatile compounds, are usually responsible for these changes (Curl and Truelove 1986). It has often been speculated that AM fungi are able to produce substances capable of interfering with microorganisms, but only as circumstantial evidence (Andrade *et al.* 1997; Linderman 1988; Paulitz & Linderman 1991). Here we present a strong evidence which shows that a crude extract of the growth medium of an AM fungus (*G. intraradices*) contains substances responsible for the stimulation or

inhibition of growth of some soil microorganisms. These results strongly suggest that the extraradical mycelium of *G. intraradices* releases substances into the environment and that these substances have an important impact on the soil microbial equilibrium. The nature of these substances is still unknown. However, given the results obtained, it is improbable that *G. intraradices* releases general inhibitory substances, such as antibiotics. The release of stimulatory substances, such as carbohydrates or soluble sugars, also seems unlikely. Wright & Upadhyaya (1996) mentioned that AM fungi are major contributors of proteins in the soil. They demonstrated that many AM fungi actively release an unusual and abundant protein that is involved in the stabilization of soil aggregates. Amino acids and proteins would represent a group of substances that can induce the kind of differential responses that we obtained.

Because the extraradical mycelium of AM fungi occupy a far greater volume of soil than roots, its action on microorganism populations may be significant and participate synergistically or antagonistically with the effect of root exudation. Many authors have shown that the microbial community is strongly modified around mycorrhizal roots as compared with non-mycorrhizal controls (Ames *et al.* 1984; Bagyaraj & Menge 1978; Bansal & Mukerji 1994; Catska 1994; Citernesi *et al.* 1996; Meyer & Linderman 1986; Nemec 1994; Posta *et al.* 1994; Secilia & Bagyaraj 1987). These studies were mainly done in pot cultures were it is not possible to differentiate between the effect of roots and of AM fungal mycelium. However, it has been shown that root exudation is modified when a plant is mycorrhizal (Bansal & Mukerji 1994; Schwab *et al.* 1984). Furthermore, the mycosphere effect may also have a strong influence in the mycorrhizosphere. By creating a new environment, with the substances released by the extraradical mycelium of AM fungi, a specific microflora can proliferate

and modify the microbial equilibrium, potentially antagonizing pathogen populations. A recent study clearly showed that the microflora present near the root system and in the mycosphere is qualitatively and quantitatively very different (Andrade *et al.* 1997). This creation of specific habitats for soil microorganisms can contribute to increase the soil biodiversity, a key element in maintaining a good soil quality and sustainability (Kennedy & Smith 1995). AM fungi can have an important role to play in sustainable agriculture, in addition to their well known nutritional effect.

St-Arnaud *et al.* (1995b) have previously reported that *G. intraradices* could directly interact with *F. o. chrysanthemi* in a similar *in vitro* system. They noted a stimulation of conidial germination of *F. o. chrysanthemi* when conidia were inoculated directly in the AM fungus compartment as compared with the non-mycorrhizal control. The fact that in our experiment, conidial germination was inhibited rather than stimulated, as reported by St-Arnaud *et al.* (1995b), likely relates to the composition of the growth medium. St-Arnaud *et al.* (1995b) used a minimal medium without sucrose and solidified with Gel Gro, while in our study, the germination medium was a crude extract from water Gel Gro. The chemical composition of the growth media have a major impact on the chemical modifications (pH, ion concentration) induced by AM fungal growth. Bago *et al.* (1996) have shown that *G. intraradices* strongly modifies the pH of the growth media, in relation with the nitrogen source. We showed that a pH range of 6·5 to 8·0 does not influence the conidial germination of *F. o. chrysanthemi.* St-Arnaud *et al.* (1995b) used a modified M medium in the distal compartment (initial pH of 5·5). It was shown that *G. intraradices* can strongly basify the pH of modified M medium locally, creating important differences between the distal compartment containing only the extraradical mycelium of *G. intraradices* (pH up to 9·0) and controls

(Villegas *et al.* 1996). It is therefore possible that the differences between the results of St-Arnaud *et al.* (1995b) and those obtained in the present experiment were caused by differences in the pH of the germination medium.

In the experiment presented here, *G. intraradices* is shown to have different effects on the growth of different microorganisms. These effects ranged from a strong stimulation (*T. harzianum*, *P. chlororaphis*) to a significant inhibition (*F. o. chrysanthemi*) of growth. In addition to the direct interactions between extraradical mycelium of AM fungi and soilborne pathogens, it is possible that more complex interactions also occur in the mycosphere. The stimulation of microorganisms that are known to have positive effects on plant growth (such as *P. chlororaphis* and *T. harzianum*), themselves could interfere with pathogens that can also have an influence on disease suppression. AM fungi, by creating a new environment in the mycosphere may contribute to the proliferation of specific microorganisms, some of them interacting with pathogens through a range of mechanisms such as antibiosis, competition for resources and parasitism.

More studies are needed to understand clearly the highly complex interactions that are implicated. Furthermore, this knowledge should eventually permit the development of mixed inoculums of AM fungi with other beneficial microorganisms that can act in a synergistic way. The comprehension of the mycorrhizosphere ecology certainly represents one of the biggest challenges of the next decades, and will serve to improve the biological control of root pathogens in a context of sustainable agriculture.

ACKNOWLEDGEMENTS

The authors wish to thank Javier Villegas, Peter Moutoglis and Brigitte Vimard for technical expertise, Stéphane Daigle for advice on experimental design and statistical analyses, and Anne Bruneau for the revision of the linguistic content of the paper. Fungal and bacterial strains were kindly provided by Dr. Yves Piché (Université Laval, Sainte-Foy, Canada), Michel Lacroix (MAPAQ, Sainte-Foy, Canada) and the company Premier Tech (Rivière-du-Loup, Canada). FCAR and NSERC operating grants to Dr. J. André Fortin supported this research.

REFERENCES

Agrios, G. N. 1997. Plant pathology, 4th ed. San Diego: Academic Press Ltd.

Ames, R. N., Reid, C. P. P., Ingham, E. R. 1984. Rhizosphere bacterial population responses to root colonization by a vesicular-arbuscular mycorrhizal fungus. New Phytol. 96: 555-563.

Andrade, G., Mihara, K. L., Linderman, R. G., Bethlenfalvay, G. J. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192: 71-79.

Azcón-Aguilar, C., Barea, J. M. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - An overview of the mechanisms involved. Mycorrhiza 6: 457-464.

Bago, B., Vierheilig, H., Piché, Y., Azcón-Aguilar, C. 1996. Nitrate depletion and pH changes induced by the extraradical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. New Phytol. 133: 273-280.

Bago, B., Azcón-Aguilar, C. 1997. Changes in the rhizospheric pH induced by arbuscular mycorrhiza formation in onion (*Allium cepa* L.). Z. Pflanzenernaehr. Bodenkd. 160: 333-339.

Bagyaraj, D. J., Menge, J. A. 1978. Interaction between a VA mycorrhiza and *Azotobacter* and their effects on rhizosphere microflora and plant growth. New Phytol. 80: 567-573.

Bagyaraj, D. J. 1984. Biological interactions with VA mycorrhizal fungi. In: Conway, L. I., Powell, C. L., Bagyaraj, D. J., eds. VA mycorrhizae. Boca Raton: CRC Press, 132-153.

Bansal, M., Mukerji, K. G. 1994. Positive correlation between AM-induced changes in root exudation and mycorrhizosphere mycoflora. Mycorrhiza 5: 39-44.

Bécard, G., Fortin, J. A. 1988. Early events of vesicular-arbuscular mycorrhiza formation on Ri T-DNA transformed roots. New Phytol. 108: 211-218.

Benhamou, N., Fortin, J. A., Hamel, C., St-Arnaud, M., Shatilla, A. 1994. Resistance responses of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum* f. sp. *chrysanthemi*. Phytopathology 84: 958-968.

Caron, M. 1989. Potential use of mycorrhizae in control of soil-borne diseases. Can. J. Plant Pathol. 11: 177-179.

Caron, M., Fortin, J. A., Richard, C. 1986. Effect of phosphorus concentration and *Glomus intraradices* on Fusarium crown and root rot of tomatoes. Phytopathology 76: 942-946.

Catská, V. 1994. Interrelationships between vesicular-arbuscular mycorrhiza and rhizosphere microflora in apple replant disease. Biol. Plant. 36: 99-104.

Citernesi, A. S., Fortuna, P., Filippi, C., Bagnoli, G., Giovannetti, M. 1996. The occurrence of antagonistic bacteria in *Glomus mosseae* pot cultures. Agronomie 16: 671-677.

Curl, E. A., Truelove, B. 1986. The rhizosphere. Giessen: Springer-Verlag.

Davies, F. T., Potter, J. R., Linderman, R. G. 1992. Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of

pepper plants independent of plant size and nutrient content. J. Plant Physiol. 139: 289-294.

Dehne, H. W. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72: 1115-1119.

Fitter, A. H., Garbaye, J. 1994. Interactions between mycorrhizal fungi and other soil organisms. Plant Soil 159: 123-132.

Gianinazzi-Pearson, V., Dumas-Gaudot, E., Gollotte, A., Tahiri-Alaoui, A., Gianinazzi, S. 1996. Cellular and molecular defense-related root responses to invasion by arbuscular mycorrhizal fungi. New Phytol. 133: 45-57.

Harrison, M. J., Dixon, R. A. 1993. Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular-arbuscular mycorrhizal associations in roots of *Medicago truncatula*. Mol. Plant-Microbe Interact. 6: 643-654.

Hooker, J. E., Jaizme-Vega, M., Atkinson, D. 1994. Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In: Gianinazzi, S., Schüepp, H., eds. Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Basel: Birkhäuser Verlag, 191-200.

Kennedy, A. C., Smith, K. L. 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant Soil 170: 75-86.

Klironomos, J. N., Ursic, M. 1998. Density-dependent grazing on the extraradical hyphal network of the arbuscular mycorrhizal fungus, *Glomus intraradices*, by the collembolan, *Folsomia candida*. Biol. Fertil. Soils 26: 250-253.

Lambais, M. R., Mehdy, M. C. 1993. Suppression of endochitinase, beta-1-3-endoglucanase, and chalcone isomerase expression in bean vesicular-arbuscular

mycorrhizal roots under different soil phosphate conditions. Mol. Plant-Microbe Interact. 6: 75-83.

Larsen, J., Jakobsen, I. 1996. Effects of a mycophagous collembola on the symbioses between Trifolium subterraneum and three arbuscular mycorrhizal fungi. New Phytol. 133: 295-302.

Lehmann, E. 1975. Nonparametrics-statistical methods based on ranks. Oakland: Holden-Day.

Linderman, R. G. 1988. Mycorrhizal interactions with the rhizosphere micoflora: the mycorrhizosphere effect. Phytopathology 78: 366-370.

Linderman, R. G., Paulitz, T. C. 1990. Mycorrhizal-rhizobacterial interactions. In: Hornby, R. J. C. D., Henis, Y., Ko, W.H., Rovira, A.D., Schippers, B., Scott, P. R., eds. Biological control of soil-borne plant pathogens. Wallingford: CAB International, 261-283.

Meyer, J. R., Linderman, R. G. 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Biol. Biochem. 18: 191-196.

Moore, J. C., St-John, T. V., Coleman, D. C. 1985. Ingestion of vesicular-arbuscular mycorrhizal hyphae and spores by soil microarthropods. Ecology 66: 1979-1981.

Nelsen, C. E., Safir, G. R. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154: 407-413.

Nemec, S. 1994. Soil microflora associated with pot cultures of *Glomus intraradix*-infected *Citrus reticulata*. Applied Soil Ecol. 1: 299-306.

Newsham, K. K., Fitter, A. H., Watkinson, A. R. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. J. Ecol. 83: 991-1000.

Paulitz, T. C., Linderman, R. G. 1989. Interactions between fluorescent pseudomonads and VA mycorrhizal fungi. New phytol. 113: 37-45.

Paulitz, T. C., Linderman, R. G. 1991. Mycorrhizal interactions with soil organisms. In: Dilip, B. R., Arora, K., Mukerij, K. G., Knudsen, G. R., eds. Handbook of Applied Mycology, vol. 1. New York: Marcel Dekker Inc., 77-129.

Posta, K., Marschner, H., Römheld, V. 1994. Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize. Mycorrhiza 5: 119-124.

Reddell, P., Spain, A. V., Hopkins, M. 1997. Dispersal of spores of mycorrhizal fungi in scats of native mammals in tropical forests of northeastern Australia. Biotropica 29: 184-192.

Remy, W., Taylor, T. N., Hass, H., Kerp, H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc. Natl. Acad. Sci. USA 91: 11841-11843.

SAS Institute 1996. The SAS system for Windows, release 6.12. Cary: SAS Institute Inc.

Schwab, S. M., Leonard, R. T., Menge, J. A. 1984. Quantitative and qualitative comparison of root exsudates of mycorrhizal and nonmycorrhizal plant species. Can. J. Bot. 62: 1227-1231.

Secilia, J., Bagyaraj, D. J. 1987. Bacteria and actinomycetes associated with pot cultures of vesicular-arbuscular mycorrhizas. Can. J. Microbiol. 33: 1069-1073.

Simon, L., Bousquet, J., Lévesque, R. C., Lalonde, M. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363: 67-69.

Smith, S. E., Gianinazzi-Pearson, V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39: 221-244.

Smith, S. E., Read, D. J. 1997. Mycorrhizal symbiosis. Cambridge: Academic Press.

Spanu, P., Boller, T., Ludwig, A., Wiemken, A., Faccio, A., Bonfante-Fasolo, P. 1989. Chitinase in roots of mycorrhizal *Allium porrum*: regulation and localization. Planta 177: 447-455.

Spanu, P., Bonfante-Fasolo, P. 1988. Cell-wall-bound peroxidase activity in roots of mycorrhizal *Allium porrum*. New Phytol. 109: 119-124.

St-Arnaud, M., Hamel, C., Caron, M., Fortin, J. A. 1994. Inhibition of *Pythium ultimum* in roots and growth substrate of mycorrhizal *Tagetes patula* colonized with *Glomus intraradices*. Can. J. Plant Pathol. 16: 187-194.

St-Arnaud, M., Hamel, C., Caron, M., Fortin, J. A. 1995a. Endomycorhizes VA et sensibilité des plantes aux maladies: synthèse de la littérature et mécanismes d'interaction potentiels. In: Fortin, J. A., Charest, C., Piché, Y., eds. La symbiose mycorhizienne - état des connaissances. Frelighsburg: Orbis Publishing, 51-87.

St-Arnaud, M., Hamel, C., Vimard, B., Caron, M., Fortin, J. A. 1995b. Altered growth of *Fusarium oxysporum* f. sp. *chrysanthemi* in an in vitro dual culture system with the vesicular arbuscular mycorrhizal fungus *Glomus intraradices* growing on *Daucus carota* transformed roots. Mycorrhiza 5: 431-438.

St-Arnaud, M., Hamel, C., Vimard, B., Caron, M., Fortin, J. A. 1996. Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an in vitro system in the absence of host roots. Mycol. Res. 100: 328-332.

St-Arnaud, M., Hamel, C., Vimard, B., Caron, M., Fortin, J. A. 1997. Inhibition of *Fusarium oxysporum* f. sp. *dianthi* in the non-VAM species *Dianthus caryophyllus* by co-culture with *Tagetes patula* companion plants colonized by *Glomus intraradices*. Can. J. Bot. 75: 998-1005.

Sutton, J. C., Sheppard, B. R. 1976. Aggregation of sand-dune by endomycorrhizal fungi. Can. J. Bot. 54: 326-333.

Taylor, T. N, Remy, W., Hass, H., Kerp, H. 1995. Fossil arbuscular mycorrhizae from the early Devonian. Mycologia 87: 560-573.

Thimm, T., Larink, O. 1995. Grazing preferences of some collembola for endomycorrhizal fungi. Biol. Fertil. Soils 19: 266-268.

Tisdall, J. M. 1994. Possible role of soil microorganisms in aggregation in soils. Plant Soil 159: 115-121.

Trappe, J. M. 1987. Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir, G. R., eds. Ecophysiology of VA mycorrhizal plants. Boca Raton: CRC Press, 5-25.

Vierheilig, H., Alt, M., Mohr, U., Boller, T., Wiemken, A. 1994. Ethylene biosynthesis and activities of chitinase and beta-1,3-glucanase in the roots of host and non-host plants of vesicular-arbuscular mycorrhizal fungi after inoculation with *Glomus mosseae*. J. Plant Physiol. 143: 337-343.

Villegas, J., Williams, R. D., Nantais, L., Archambault, J., Fortin, J. A. 1996. Effects of N source on pH and nutrient exchange of extramatrical mycelium in a mycorrhizal Ri T-DNA transformed root system. Mycorrhiza 6: 247-251.

Volpin, H., Elkind, Y., Okon, Y., Kapulnik, Y. 1994. A vesicular arbuscular mycorrhizal fungus (*Glomus intraradix*) induces a defense response in alfalfa roots. Plant Physiol. 104: 683-689.

Wright, S. F., Franke-Snyder, M., Morton, J. B., Upadhyaya, A. 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant Soil 181: 193-203.

Wright, S. F., Upadhyaya, A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161: 575-586.

TABLES

Table 1. Effect of *Glomus intraradices* extraradical mycelium and concentration on the pH of a crude extract of the growth medium.

Extract concentration factor	*G. intraradices*		Control	
	pH[1]	SD[2]	pH	SD
1·0	7·38 a[3]	0·29	7·51 a	0·24
2·5	7·25 a	0·32	7·43 a	0·26
5·0	7·10 a	0·29	7·36 b	0·27
10·0	6·58 a	0·30	7·16 b	0·20

[1]Values are means of 18 determinations of pH.

[2]Standard deviation of the mean.

[3]For each concentration factor, pH values followed by a different letter are significantly different using a Tukey's studentized range test ($P < 0·05$).

Table 2. Effect of the pH extract on conidial germination of *Fusarium oxysporum* f. sp. *chrysanthemi* and *Trichoderma harzianum.*

Fungal species	pH							
	6·5		7·0		7·5		8·0	
	%[1]	SD[2]	%	SD	%	SD	%	SD
F. o. chrysanthemi[3]	86·6a	4·5	85·7a	4·3	85·5a	4·7	86·6a	4·2
T. harzianum[4]	64·6a	6·5	62·4a	5·0	63·4a	6·7	64·3a	6·6

[1]Values are means of 24 repetitions. Within each line, percentages followed by the same letter are not significantly different using a Tukey's studentized range test ($P < 0.05$).

[2]Standard deviation of the mean.

[3]After incubation at 22°C during 6 h.

[4]After incubation at 22°C during 14 h.

Table 3. Effect of the pH extract on growth of *Pseudomonas chlororaphis* and *Clavibacter michiganensis* subsp. *michiganensis*.

Bacterial species	pH							
	6·5		7·0		7·5		8·0	
	Growth[1]	SD[2]	Growth	SD	Growth	SD	Growth	SD
P chlororaphis[3]	-0·38a	0·43	-0·09b	0·76	-0·12b	0·40	-0·23a	0·51
C. m. michiganensis[4]	-0·30a	0·23	-0·22a	0·24	0·21b	0·68	0·32b	0·13

[1]Values are means of 24 repetitions and represent the growth of bacterial population as estimated by the difference between final and initial O.D. values. Within each line, values followed by a different letter are significantly different using a Tukey's studentized range test ($P < 0·05$).

[2]Standard deviation of the mean.

[3]After incubation at 28°C during 24 h.

[4]After incubation at 28°C during 48 h.

FIGURES

LEGEND OF FIGURES

Figure 1. Effect of a crude extract of the growth medium of *Glomus intraradices* on conidial germination (%) of *Fusarium oxysporum* f. sp. *chrysanthemi*. Values are the mean of 35 replicates. For each concentration, germination percentages with an asterisk (*) indicate a significant difference by ANOVA ($P<0{\cdot}05$) between AM fungus extract and control extract. In presence of the AM fungus extract (a,b,c,d) or in presence of the control extract (w,x,y,z), germination percentages with a different letter are significantly different by a Tukey's studentized range test ($P < 0{\cdot}05$).

Figure 2. Effect of a crude extract of the growth medium of *Glomus intraradices* on conidial germination (%) of *Trichoderma harzianum*. Values are the mean of 35 repetitions. For each concentration, germination percentages with an asterisk (*) indicate a significant difference by ANOVA ($P<0{\cdot}05$) between AM fungus extract and control extract. In presence of the AM fungus extract (a,b) or in presence of the control extract (w,x,y), germination percentages with a different letter are significantly different by a Tukey's studentized range test ($P < 0{\cdot}05$).

Figure 3. Effect of a crude extract of the growth medium of *Glomus intraradices* on bacterial growth of *Pseudomonas chlororaphis*. Values are the mean of 18 repetitions. For each concentration, values represent the bacterial growth as estimated by the difference between final and initial O.D. values. Germination percentages with an

asterisk (*) indicate a significant difference by ANOVA (P<0·05) between AM fungus extract and control extract. In presence of the AM fungus extract (a,b,c,d) or in presence of the control extract (w,x), germination percentages with a different letter are significantly different by a Tukey's studentized range test (P < 0·05).

Figure 4. Effect of a crude extract of the growth medium of *Glomus intraradices* on bacterial growth of *Clavibacter michiganensis* subsp. *michiganensis*. Values are the mean of 30 repetitions. For each concentration, values represent the bacterial growth as estimated by the difference between final and initial O.D. values. Germination percentages with an asterisk (*) indicate a significant difference by ANOVA (P<0·05) between AM fungus extract and control extract. In presence of the AM fungus extract (a,b,c) or in presence of the control extract (w,x,y), germination percentages with a different letter are significantly different by a Tukey's studentized range test (P < 0·05).

Figure 1.

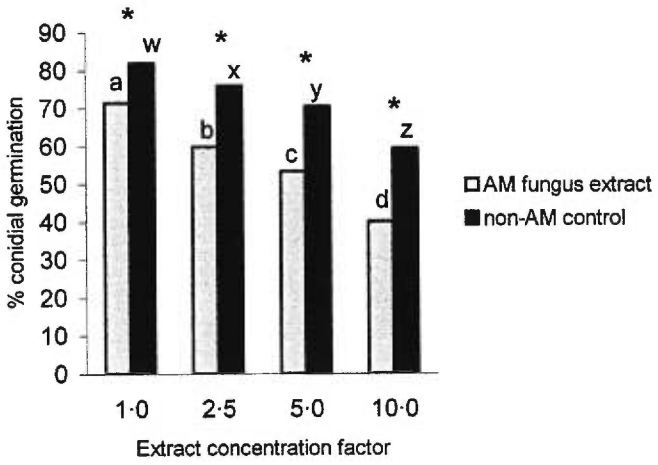

% conidial germination
90
80
70
60
50
40
30
20
10
0
a
b
c
d
w
x
y
z
*
1·0
2·5
5·0
10·0
Extract concentration factor
AM fungus extract
non-AM control

Figure 2.

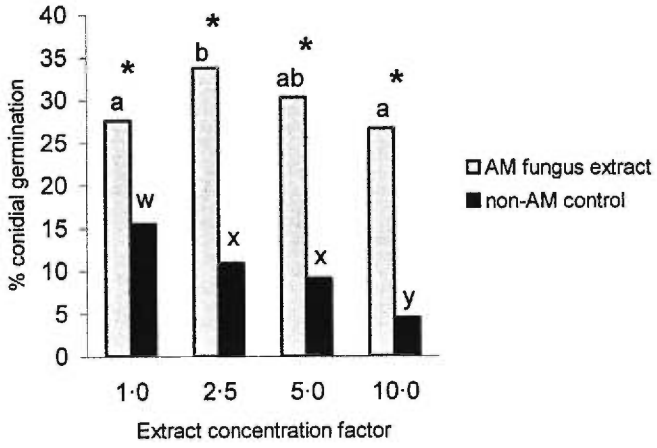

40
35
30
25
20
15
10
5
0
% conidial germination
*
a
w
*
b
x
*
ab
x
*
a
y
1·0
2·5
5·0
10·0
Extract concentration factor
AM fungus extract
non-AM control

Figure 3.

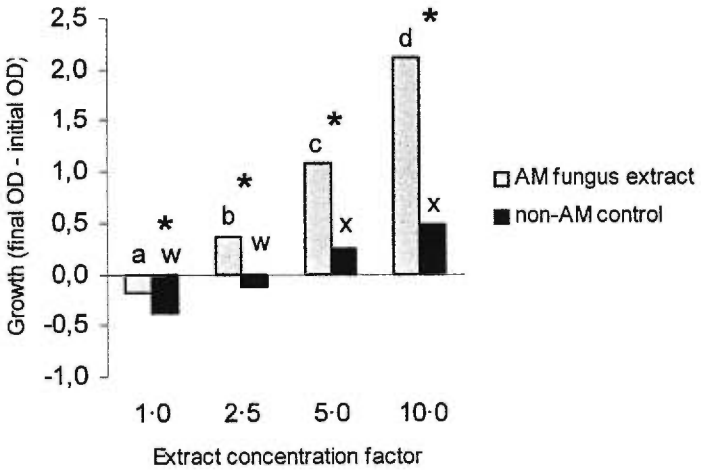
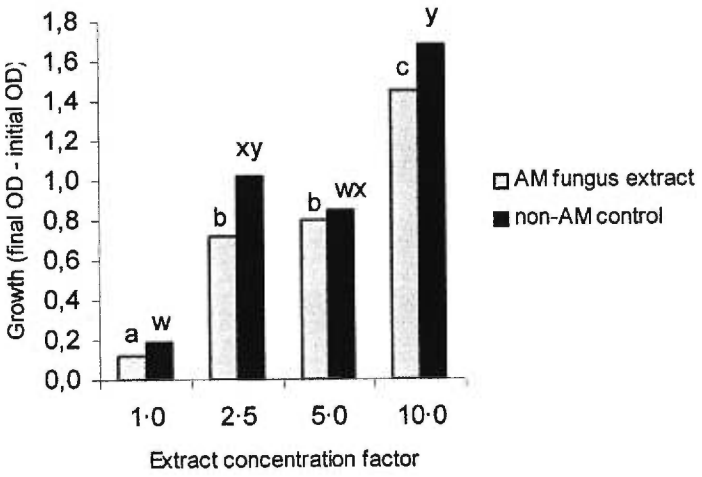

2,5
2,0
1,5
1,0
0,5
0,0
-0,5
-1,0
Growth (final OD - initial OD)
a
w
*
b
w
*
c
x
*
d
x
*
1·0
2·5
5·0
10·0
Extract concentration factor
AM fungus extract
non-AM control

Figure 4.


Growth (final OD - initial OD)
1,8
1,6
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
a
w
b
xy
b
wx
c
y
1·0
2·5
5·0
10·0
Extract concentration factor
AM fungus extract
non-AM control

3. BIOLOGICAL CONTROL OF *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* POPULATIONS BY *GLOMUS INTRARADICES*

Ce manuscrit, ayant comme coauteurs: M. Filion, M. St-Arnaud and J. A. Fortin, sera soumis à la revue Phytopathology. Ce travail traite de la dynamique de populations de micro-organismes, notamment du *Clavibacter michiganensis* subsp. m*ichiganensis*, sous l'influence du *Glomus intraradices* en sol. Ce suivit est réalisé grâce à l'utilisation de la technologie du DAS-ELISA ainsi que d'outils classiques. Cette étude effectuée en conditions de sol à l'aide de systèmes expérimentaux simplifiés permet de corroborer les résultats obtenus au chapitre précédent mais en milieu plus complexe. Cette étude démontre clairement la capacité de contrôle biologique que le *Glomus intraradices* possède face au *Clavibacter michiganensis* subsp. *michiganensis*.

RÉSUMÉ

Un système de culture compartimenté fut développé afin d'investiguer la dynamique du *Clavibacter michiganensis* subsp. *michiganensis* et d'autres groupes bactériens dans la mycorhizosphère de plants de tomates avec ou sans la présence du champignon endomycorhizien arbusculaire (MA) *Glomus intraradices*. Ce système a permis de distinguer entre des effets rhizosphériques et des effets mycosphériques du champignon MA sur la population du *C. m. michiganensis* en sol. Une approche spécifique et quantitative à l'aide de la technologie du DAS-ELISA fut développée. À l'aide d'un anticorps polyclonal anti-*C. m. michiganensis*, nous avons démontré que la population du *C. m. michiganensis* fut significativement réduite dans la rhizosphère de plants de tomates par comparaison à la population distante des racines. De plus, la population du *C. m. michiganensis* fut significativement réduite par la présence de racines de tomates colonisées par le *G. intraradices* comparé aux racines non-mycorhiziennes. La présence du *G. intrardices* n'eu pas d'effet significatif sur la population des bactéries Gram-. Par contre, la présence du champignon MA stimula significativement la présence des *Pseudomonas fluorescens*. En réduisant directement la présence du *C. m. michiganensis* et en augmentant la présence des *Pseudomonas fluorescens*, qui sont reconnus pour leur capacité à stimuler la croissance des plantes ainsi que leur pouvoir antagoniste face à divers parasites, *G. intraradices* a démontré un bon potentiel de contrôle biologique face au *C. m. michiganensis*.

Mots-clés : champignons MA, *Glomus intraradices*, DAS-ELISA, mycorhizosphère.

SUMMARY

A compartmentalized growth system unit was developed to investigate the dynamic of *Clavibacter michiganensis* subsp. *michiganensis* and other bacteria in the mycorrhizosphere of tomato plants with or without the presence of the arbuscular mycorrhizal (AM) fungus *Glomus intraradices*. This system allowed to distinguish between the rhizosphere effect and the AM mycosphere effect on *C. m. michiganensis* population in the soil. A specific quantitative DAS-ELISA approach, using a polyclonal anti-*C. m. michiganensis* antibody, revealed that the population of *Clavibacter michiganensis* subsp. *michiganensis* was significantly reduced in the rhizosphere of tomato plants as compared with bulk soil. Furthermore, the population of *C. m. michiganensis* was significantly decreased by the presence of tomato roots colonized by *Glomus intraradices* as compared to non-mycorrhizal roots. The presence of *G. intraradices* had no significant effect on total population of Gram- bacteria. However, the presence of the AM fungus significantly enhanced the presence of fluorescent Pseudomonads. By directly reducing the presence of *C. m. michiganensis* and increasing total fluorescent Pseudomonads number, which are known to stimulate plant growth and antagonize many soil pathogens, *Glomus intraradices* showed a good biocontrol potential against *C. m. michiganensis*, the causal agent of tomato bacterial canker.

Key words : AM fungi, *Glomus intraradices*, DAS-ELISA, mycorrhizosphere.

INTRODUCTION

Bacterial canker of tomato (*Lycopersicon exculentum* Mill.), caused by *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al., is of significant economic importance in commercial tomato production worldwide (Jones *et al.* 1991). Since the 1930s, research on seed treatments for bacterial canker control has led to the development of many techniques to reduce contamination. For example, chemicals like copper-containing bactericides were used in an attempt to reduce the incidence of the disease. However, the pathogen remains a major problem in many tomato productions and research is needed to improve the detection and protection against *C. m. michiganensis* (Gleason *et al.* 1993). The failure to produce tomato cultivars with significant resistance or tolerance to bacterial canker reinforces the interest to develop a biological control approach to reduce disease incidence. However, the application of a biocontrol agent, known to have a strong negative impact on one specific pathogen, has often given inconsistent results in agrosystems conditions (Cook 1986; Weller 1997). The discovery of an antagonizing microorganism under *in vitro* conditions does not necessarily imply that this microorganism possess a good rhizosphere competence under field or greenhouse conditions, where many microorganisms are present and interact with each others (Papavizas & Lumsden 1980; Whipps 1997). For these reasons, no biocontrol agent has proven to be significantly efficient against *C. m. michiganensis* in soil until now.

A more holistic approach aims at the creation of a specific environment unfavorable to *C. m. michiganensis* but favorable to microorganisms beneficial to the plant and antagonistic to the pathogen. Arbuscular mycorrhizal (AM) fungi have the

potential to achieve this goal. They are known to have a general beneficial impact on plant growth (Smith & Gianinazzi-Pearson 1988; Smith & Read 1997), water stress resistance (Davies *et al.* 1992; Nelsen & Safir 1982), soil aggregation (Schreiner & Bethlenfalvay 1995; Sutton & Sheppard 1976; Tisdall 1994; Wright & Upadhyaya 1996) and soilborne disease reduction (Azcón-Aguilar & Barea 1996; Bagyaraj 1984; Caron 1989; Dehne 1982; Hooker *et al.* 1994; St-Arnaud *et al.* 1995). These organisms are candidates to become good biocontrol agents and, by creating a new environment named the mycorrhizosphere (Linderman 1988), can also favour good edaphic conditions beneficial to the plant (Hooker & Black 1995). AM fungi are known to have the ability to reduce pathogen populations in the mycorrhizosphere (Caron *et al.* 1986; Catska 1994; Garcia-Garrido & Ocampo 1988; Garcia-Garrido & Ocampo 1989; Hwang *et al.* 1992; Kaye *et al.* 1983; Krishna & Bagyaraj 1982; McAllister *et al.* 1994; St-Arnaud *et al.* 1994; St-Arnaud *et al.* 1997; Wacker *et al.* 1990). Furthermore, they can stimulate the growth of other microorganisms known to have a beneficial influence on plant growth and protection (Catska 1994; Citernesi *et al.* 1996; Meyer & Linderman 1986; Secilia & Bagyaraj 1987).

Until now, studies investigating the plant-microorganisms or microorganisms-microorganisms interactions in the mycorrhizosphere used classical quantitative techniques such as plating on semi- or selective media or total microscopic bacterial or fungal counts. Few selective media were developed in an attempt to isolate *C. m. michiganensis* from soil (Gleason *et al.* 1993; Shirakawa & Sasaki 1988). Only one is reported to give good results (Shirakawa & Sasaki 1988). The development of more accurate molecular quantitative techniques such as quantitative polymerase chain reaction (PCR) or quantitative enzyme link immunosorbent assay (ELISA) now permit

to investigate with more precision the dynamic of certain specific microorganisms under different conditions (Akkermans *et al.* 1995; Schloter *et al.* 1995; Singh & Singh 1995). The availability of a commercial specific polyclonal antibody against *C. m. michiganensis* (Agdia Inc, Elkhart, IN, USA) promoted the use of ELISA to detect this pathogen in diseased plant parts (Gitaitis *et al.* 1991). However, few studies showed the use of the ELISA technology to quantify specific microorganisms directly extracted from soil (Dewey *et al.* 1997; Otten *et al.* 1997; Schloter *et al.* 1995; Thornton *et al.* 1993). We therefore adapted protocols of bacterial extraction in order to use quantitative double antibody sandwich (DAS)-ELISA as a new tool for specific and accurate detection of *C. m. michiganensis* in soil. The specific objectives of the current study were to (i) develop a new concept of growth units simulating natural mycorrhizosphere conditions; (ii) adapt the DAS-ELISA technology to quantify *C. m. michiganensis* directly isolated from soil; (iii) investigate the dynamic of *C. m. michiganensis* and of other ecologically important groups of bacteria in different regions of the mycorrhizosphere of tomato plants; and (iv) determine the biological control potential of *G. intraradices* against *C. m. michiganensis*.

MATERIALS AND METHODS

Experimental systems

Growth units were developed for the purpose of this research (Fig. 1). Each of them consisted of an assemblage of four standard polystyrene centrifuge tubes (three 15 ml and one 50 ml). The central compartment of the system was made with the 50 ml tube, cut to remove the closed end. Three circular holes were made (2 cm in diameter), each at 2 cm from the lower end of the 50 ml tube and at equal distance around the circumference. Three 15 ml tubes were cut to obtain 6 cm length cylinders with open extremities. For each modified 15 ml tubes, two pierced caps were glued together with nylon or mixed cellulose esters membranes between each and placed on each tube. The membrane porosity used were respectively 750 µm and 40 µm for the nylon membranes (B & SH Thompson, Ville Mont Royal, Québec, Canada) and 0.45 µm for the mixed cellulose esters membrane (Millipore, Nepean, Ontario, Canada). The other extremities of the modified 15 ml tubes were closed with standard test tube caps. The three modified 15 ml tubes were fixed to the central compartment facing the 2 cm holes. The 750 µm membrane separated the root compartment from the central compartment; the 40 µm membrane separated the hyphal compartment from the central compartment; and the 0.45 µm membrane separated the bulk soil compartment from the central compartment. The cap of the central compartment was fixed on the lower extremity of the 50 ml tube and the growth units obtained were painted black to avoid penetration of the light in the compartments.

Soil and mycorrhizal inoculation

Sandy soil from an agricultural field (90% sand, 9% silt, 1% clay; and 4.1% organic matter [pH 6.0]) was sieved (2-mm-pore-sized) and stored at 4°C. Nutrient Long Ashton solution solution was added at a rate of 140 ml (Hewitt 1966) per kilogram of soil. The soil was then autoclaved at 121°C for 60 min, three times on three consecutive days. Each growth unit received 63.0 g (fw) of soil/central compartment and 14.5 g (fw) of soil/small compartment (root, hyphal and bulk soil compartments). In order to reintroduce microorganisms other than AM fungi, all growth units received 6 ml (3 ml in the central compartment and 1 ml per small compartment) of a soil filtrate obtained from a suspension of 100 g of soil in 1 l of distilled water, macerated for 10 min and filtered through a # 1 filter paper (Whatman).

Mycorrhizal leek (*Allium porrum* L.) roots, colonized with *Glomus intraradices* Schenck & Smith were obtained from 20 month-old pot cultures and served as mycorrhizal inoculum. They were surface sterilized for 20 min with a 2% (w/v) chloramine-T solution and 0.02% (w/v) streptomycin sulphate. The central compartment received 1.5 g of mycorrhizal roots as inoculum (myc+). Controls (myc-) in the central compartment received 1.5 g of mycorrhizal roots autoclaved at 121°C for 60 min.

Plants, growth conditions and bacteria inoculation

Tomato seeds (*Lycopersicon esculentum* Mill.) cultivar Jet Star (Willhite Seed Inc., Poolville, Texas, USA) were washed with 70% (v/v) ethanol solution for 30 sec, rinsed with sterile distilled water, surface sterilized in 1.8% (v/v) sodium hypochlorite

solution for 20 min and rinsed again with sterile distilled water. Two seeds were sown per growth unit and thinned to one after seedling emergence. Twenty-four growth units (12 myc+ and 12 myc-) were prepared and maintained in a growth chamber at 22°C (day) and 16°C (night), with 60-70% relative humidity. Fluorescent lamps were operated 16 h · d^{-1} and generated a photon flux of 300 µmol · m^{-2} · s^{-1} (PAR). The plants were watered daily in the central compartment with sterile distilled water and randomized weekly inside the growth chamber. After 3 weeks of growth, 3 ml of Long Ashton solution was added to the central compartment. After 5 weeks of growth, 1 ml of a bacterial suspension of *C. m. michiganensis* (8×10^9 bact. · ml^{-1}) was inoculated to each of the three small compartments of the growth units through the end cap by the use of a syringe. Eight growth units (4 myc+ and 4 myc-) were randomly removed from the growth chamber for analysis 12 h, 36 h and 60 h after inoculation of the bacterial canker pathogen. The soil from each of the three small compartments was harvested separately and thouroughly mixed in order to uniformise the microorganism content within the sample.

Shoot dry weight, soil water content and arbuscular colonization

Shoots were harvested from each system and dried at 70°C for 48 h for shoot dry weight determination. The water content of the soil was determined from a 1 g sample after drying at 70°C for 48 h. The roots of each plant were removed from the central compartment, cut in 1-cm sections and stained with acid fuchsin (Kormanik & McGraw 1982). The percentage of root length colonized by *G. intraradices* was determined using the gridline intersect method (Giovannetti & Mosse 1980).

Root length, hyphal presence and soil pH

Roots were removed from the root compartment and their total length was measured on millimetric paper. Soil (5 g) was removed from root, hyphal and bulk soil compartments for quantification of the hyphae present. Each 5 g sample soil was mixed on a rotary shaker at 300 RPM in 50 ml centrifuge tubes with 40 ml of a 50% (w/v) sucrose solution (maintained at 4°C) for 30 min. The tubes were centrifuged at 700 g for 10 min at 4°C. The supernatant was filtered on # 1 filter paper (Whatman) in a funnel under vacuum pump. Each sample was processed twice and hyphae were recuperated on the same filter paper. 1 ml of a 0.2% (w/v) acid fuschin solution was added on each filter paper for 15 min, rinsed with distilled water and the presence of hyphae was quantified under a dissecting microscope in 1 cm^2 of the filter paper. A millimetric acetate was put over the filter paper and the presence of hyphal fragments was determined for each of the 100 mm^2. Results were expressed as percentage of hyphal presence per cm^2.

A 2 g soil samples was harvested separately from root, hyphal and bulk soil compartments and placed in 15 ml tubes for pH determination. 4 ml of 0.01 M $CaCl_2$ were added and the tubes were agitated for 30 min at high speed. The pH was measured by inserting a micro-electrode in the solution obtained.

Bacterial extraction from soil

Bacteria were extracted from the soil of root, hyphal and bulk soil compartments using a technique based on Bakken and Lindahl (1995). 1 g soil sample was added to 9 ml of distilled water at 4°C in a 15 ml centrifuge tube. A drill gun was equipped with a

rounded rubber pestle fixed on a 15 cm rod. The rubber pestle was designed to fit standard 15 ml test tubes with a clearing of about 0.5 to 1 mm. The soil sample was homogenized in sterile distilled water by pumping the pestle up and down while rotating at maximum speed for 2.5 min. The lower end of the tube was placed in crushed ice during homogenization. The tube was then centrifuged at 700 g for 15 min at 4°C and 7 ml of supernatant was removed from the tube and conserved at 4°C. 100 µl of extract were diluted in 900 µl extraction buffer ((w/v) 0.13% Na_2SO_2, 2.0% Polyvinylpyrrolidine, 0.02% NaN_3, 0.2% Powdered egg (chicken) albumin Grade II, 2.0% Tween-20, dissolved in 1000 ml 1X PBST (0.8% NaCl, 0.115% Na_2HPO_4, 0.02% KH_2PO_4, 0.02% KCl, 0.05% Tween-20, pH 7.4)) and was used for the ELISA tests.

Bacterial culture, specificity tests and standard curve

Clavibacter michiganensis subsp. *michiganensis* cultures were maintained on nutrient agar at 28°C in the dark. A bacterial suspension and serial dilutions up to 10^{-8} were made in distilled water. The absorbance of each dilution was read on an Emax microplate reader (Molecular devices, Menlo Park, CA, USA). The dilutions were plated on nutrient agar to obtain colony forming units (CFU) values. A specific polyclonal antibody against *C. m. michiganensis* was obtained from Agdia Inc. (Elkhart, IN, USA) and DAS-ELISA was performed (see below) for each dilution of the *C. m. michiganensis* suspension. The values obtained were plotted against CFU values and a standard curve was prepared to determine the detection limit of *C. m. michiganensis* in pure culture (Fig. 2). Since no methods are clearly recommended for establishing the

positive-negative threshold of ELISA data (Sutula *et al.* 1986), we used a standard positive threshold of 2 x negative mean.

To assure specificity of the antibody on bacteria directly extracted from soil, serial dilutions of up to 10^{-8} of *C. m. michiganensis* were made in sterile distilled water and added to the same soil used in the growth units. A 1 ml sample from each dilution, was added to 14.5 g of soil and incubated at 22°C for 12 h. Bacterial extraction and DAS-ELISA were performed as described previously to determine the detection limit of *C. m. michiganensis* in inoculated and control soil. For interpreting ELISA data, a positive threshold of 2 x negative mean was used.

DAS-ELISA protocol

First layer : MaxiSorp Nunc-Immuno plates (Nalge Nunc International, Rochester, NY, USA) were coated with 100 µl of the anti-*C. m. michiganensis* (Agdia Inc.) diluted 1:200 in the coating buffer ((w/v) 0.159% Na_2CO_3, 0.293% $NaHCO_3$, 0.02% NaN_3, distilled water, pH 9.6) and agitated at 150 RPM overnight at 4°C. Second layer : 100 µl extracted samples from soil and diluted 1:10 in the extraction buffer were added and incubated with an agitation of 150 RPM for 2 h at room temperature. Standards of *C. m. michiganensis* were supplied by Agdia Inc. Third layer : 100 µl alkaline phosphatase conjugated antibody anti-*C. m. michiganensis*, diluted 1:200 in ECI buffer ((w/v) 0.2% BSA, 2.0% Polyvinylpyrrolidone, 0.02% NaN_3, 1X PBST, pH 7.4) were incubated with an agitation of 150 RPM for 2 h at room temperature. All washing steps were done with PBST buffer. Staining was performed using 100 µl of 5 mg PNP tablets dissolved in 5 ml PNP buffer ((w/v) 0.01% $MgCl_2$, 0.02% NaN_3, (v/v)

9.7% Diethanolamine, distilled water; pH 9.8). The plates were incubated with an agitation of 150 RPM for 1 h at room temperature. 50 µl of a 3 M NaOH solution was added to block color development and the plates were read for absorbance values at 405 µm using an Emax microplate reader (Molecular devices, Menlo Park, CA, USA). Absorbance values obtained for *C. m. michiganensis* were converted from absorbance/1 g fresh soil weight to absorbance/1 g dry soil weight. This transformation was necessary because variations in soil water content up to 20% were observed between different compartment.

Estimation of bacterial population

Serial dilutions from 1 g samples from root, hyphal and bulk soil compartments were made in one-quarter strength Ringer's solution (0.05% agar) at 4°C for 8 growth units after 60 h incubation with *C. m. michiganensis* (4 myc+ and 4 myc-). Plating was done on tryptic soy agar (TSA) for total bacterial counts (Martin 1975), TSA + 0.02% (w/v) crystal violet for Gram- bacteria counts (Rovira *et al.* 1974), and King B agar + novobiocin-penicillin-cycloheximide-chloramphenicol (NPCC) for fluorescent pseudomonads counts (Simon *et al.* 1973). The plates were incubated in the dark at 25°C for three days before evaluating colony numbers.

Statistical analyses

Statistical analyses were done with Correlation and General Linear Model procedures of the SAS statistical software (SAS Institute Inc. 1996). The following

variables were analysed by ANOVA: shoot dry weight, root length AM colonization, hyphal density in soil, soil pH, *C. m. michiganensis* absorbance values · g^{-1} dry soil weight, total bacteria CFU, rank transformed Gram- bacteria CFU, rank transformed fluorescent pseudomonads CFU. A rank transformation (Lehmann 1975) was performed when needed in order to meet the ANOVA requirements. A posteriori comparison between treatments were done by Tukey's studentized range tests for the following variables: root lenght AM colonization, hyphal density in soil, *C. m. michiganensis* absorbance values · g^{-1} dry soil weight and rank transformed fluorescent pseudomonads CFU.

RESULTS

Plant growth and arbuscular mycorrhizal colonization. Shoot dry weight and root length of tomato plants were not significantly modified by *G. intraradices* inoculation (Table 1 and 2). All non-AM inoculated plants were free of mycorrhizal structures and all AM inoculated plants showed the presence of AM hyphae, vesicles and arbuscules (Table 1).

Hyphal density and soil pH. No roots were found in bulk soil or hyphal compartments. Systems without roots in the root compartment were rejected for DAS-ELISA quantification of *C. m. michiganensis* or CFU determination. Hyphae were significantly more abundant in root and hyphal compartment sas compared to the bulk soil compartment ($P<0.05$) and were significantly more abundant in mycorrhizal as compared to non-mycorrhizal systems (Table 2) ($P<0.001$). No significant pH differences were obtained between compartments or mycorrhizal treatments (Table 2).

Specificity tests and standard curve. The standard curve revealed, for pure cultures of *C. m. michiganensis*, a detection limit of 4×10^6 bacteria ml^{-1} with a positive threshold of 2 x negative mean (Fig. 2). In soil, after 12 h incubation at 22°C, *C. m. michiganensis* was recovered with an initial inoculation detection limit of 6×10^6 bacteria ml^{-1} soil extract with a positive threshold of 2 x negative mean. *Clavibacter michiganensis* subsp. *michiganensis* was not detected in control soil not previously inoculated with the bacteria.

DAS-ELISA. The quantification of *C. m. michiganensis* was investigated for each compartment and mycorrhizal treatments, for the three designed times (12 h, 36 h and 60 h). As the incubation time did not influence the population of *C. m. michiganensis*, the

data from 12 h, 36 h and 60 h incubation periods were pooled for further analysis. Absorbance values were significantly reduced in the root compartment as compared to the bulk soil compartment ($P<0.05$) (Fig. 3). No significant differences were observed between compartments in the absence of *G. intraradices*. Absorbance values were significantly reduced in the root compartment containing mycorrhizal roots as compared to the root compartment containing non-mycorrhizal roots ($P<0.05$) (Fig. 3).

Bacterial populations. Total bacterial number was not significantly influenced by the compartmentation treatments and no significant mycorrhizal effect was observed (Fig. 4).

Total Gram- bacterial number was not significantly different between compartmentation treatments and no significant mycorrhizal effect was observed (Fig. 5).

Total numbers of fluorescent Pseudomonads was significantly different between compartmentation treatments ($P<0.05$) and significantly different between mycorrhizal and non-mycorrhizal systems ($P<0.01$). A highly significant mycorrhizal*compartment interaction was also obtained ($P<0.001$). Total fluorescent Pseudomonad number was significantly higher in the presence of *G. intraradices* ($P<0.01$) and significantly lower in root compartments as compared to bulk soil compartments ($P<0.05$) (Fig 6.). Further analysis revealed that in the presence of *G. intraradices*, total fluorescent Pseudomonad number was significantly decreased in root compartments as compared to bulk soil and hyphal compartments ($P<0.001$) (Fig. 6). In the absence of *G. intraradices*, total fluorescent Pseudomonad number was significantly decreased in hyphal compartments as compared to bulk soil compartments ($P<0.05$) (Fig. 6). In hyphal compartments, total

fluorescent Pseudomonad number was significantly decreased in the absence of *G. intraradices* ($P<0.05$) (Fig. 6).

DISCUSSION

The results showed that *Glomus intraradices* has a good biocontrol potential against *Clavibacter michiganensis* subsp. *michiganensis*. In the presence of mycorrhizal roots, the amount of *C. m. michiganensis* is significantly reduce as compared to systems with non-mycorrhizal roots. However, the presence of the AM fungus alone (hyphal compartment) did not reduce the presence of *C. m. michiganensis*. Therefore, it seems that the reduction of *C. m. michiganensis* population is caused by a modification of root exsudates induced by AM colonization rather than by a direct interaction between the AM fungus and the pathogen.

The presence of *G. intraradices* did not modify the total bacterial population nor the Gram- number in any of the compartments investigated. However, *G. intraradices* significantly enhanced the number of fluorescent pseudomonad present. The highest increase in number of fluorescent pseudomonad present was observed in the hyphal compartment and was mediated by the presence of the AM fungal mycelium. In the root compartment, the number of fluorescent pseudomonads was significantly reduced as compared to the bulk soil and hyphal compartments. Fluorescent pseudomonads are known to have a general negative impact on soil pathogen populations (Nehl *et al.* 1997; Weller 1988). The ability to increase the number of fluorescent Pseudomonads present represents another beneficial effect of the establishment of this AM fungal symbiosis. In addition to pathogen reduction, PGPR, such as fluorescent Pseudomonads, are also known to improve plant growth (Weller 1988). The soil pH was not modified and therefore cannot be responsible for the results obtained. If nutritional factors were implicated, they did not modify shoot dry weight of tomato plants. Therefore,

modification of AM root exsudates, as compared to non-AM roots, remains the main hypothesis for the reduction of *C. m. michiganensis*.

It is well demonstrated that root exsudates strongly modify the microbial composition of the rhizosphere. Different substances released by the roots such as soluble sugars, phenolics, organic acids, amino acids, antibiotics and volatile compounds, are usually responsible for these changes (Curl & Truelove 1986). It has also been showed that root exsudates are modified when a plant becomes mycorrhizal (Bansal & Mukerji 1994; Schwab *et al.* 1984). However, a direct relationship between root exsudates modification and pathogen reduction had never been clearly showed before. Most of the studies showing pathogens population reductions were done in pot culture where the AM hyphal effect versus the root effect were impossible to distinguished.

The development of a new growth unit allowing the separation of the mycorrhizosphere important regions permitted us to investigate fine interactions in soil. In comparison with other systems developed to investigate the mycorrhizosphere (Addy *et al.* 1994; Joner & Jakobsen 1995; Kabir *et al.* 1997; Larsen & Jakobsen 1996; Redecker *et al.* 1995), our approach has the advantage of physically separating the three important zones of the mycorrhizosphere (root, hyphal and bulk soil), contains smaller amount of soil allowing the use of a true quantitative approach and permiting an easy recovery of each compartment. The use of a compartmentalized systems is certainly a good approach to investigate these kinds of interactions in order to differentiate the possible mechanisms implicated.

Our results also showed that the DAS-ELISA technology is a good tool to investigate the dynamics of specific microorganisms in soil. However, this technique

requires a minimum concentration of microorganisms in order to overcome the limit of detection. The limit of detection obtained (4×10^6 bacteria · ml^{-1}) is in accordance with the general detection limit of 10^6 bacteria · ml^{-1} reported (Schloter *et al.* 1995). The use of a monoclonal antibody with a higher affinity against a cell surface antigen would have permitted to lower the detection limit. A detection limit of 10^2 bacteria · ml^{-1} has been reported with the use of this technique (Schloter *et al.* 1992). However, for the purpose of this study, higher levels of detection where not necessary since a high numbers of *C. m. michiganensis* are generally present in infected sites (Gleason *et al.* 1991). In order to investigate the biocontrol potential of *G. intraradices* against *C. m. michiganensis*, conditions of infection similar to field conditions had to be used.

It is now well demonstrated that AM fungi have a strong biocontrol potential against a wide range of pathogens (Azcón-Aguilar & Barea 1996; Bagyaraj 1984; Caron 1989; Dehne 1982; Hooker *et al.* 1994; Jalali & Jalali 1991; Paulitz & Linderman 1991; St-Arnaud *et al.* 1995). The mechanisms implicated are still not fully *understood* (Azcón-Aguilar & Barea 1996; St-Arnaud *et al.* 1995). Other than direct interactions between AM fungi and pathogens, indirect effects through modification of the microflora, plant defence mechanisms activation and nutritional effects, we also showed that AM impact on root exsudation can count among mechanisms implicated. It seems that depending on plant factors, AM fungi, pathogens, and soil conditions, many of these mechanisms are likely to be involved to different degrees to improve biocontrol of root pathogens (St-Arnaud *et al.* 1995). More studies will have to be conducted to understand clearly the highly complex interactions that are implicated and to use efficiently AM fungi as biocontrol agents under greenhouse and field conditions.

ACKNOWLEDGEMENTS

The authors wish to thank Brigitte Vimard, Magda Quirion and Véronique Laflamme for technical help, and Stephane Daigle for advice on statistical analysis. The strain of *C. m. michiganensis* (MAPAQ #1059) was kindly provided by Michel Lacroix (MAPAQ, Sainte-Foy, Canada). This research was supported by FCAR and NSERC operating grants to Dr. J. André Fortin.

REFERENCES

Addy, H. D., Schaffer, G. F., Miller, M. H., Peterson, R. L. 1994. Survival of the external mycelium of a VAM fungus in frozen soil over winter. Mycorrhiza 5: 1-5.

Akkermans, A. D. L., van Elsas, J. D., De Bruijn, F. J. 1995. Molecular microbial ecology manual. Dordrecht: Kluwer Academic Publishers.

Azcón-Aguilar, C., Barea, J. M. 1996. Arbuscular mycorrhizas and biological control of soil-borne pathogens - an overview of the mechanisms involved. Mycorrhiza 6: 457-464.

Bagyaraj, J. 1984. Biological interactions with VA mycorrhizal fungi. In: Conway, L. I., Powell, C. L., Bagyaraj, J. eds. VA mycorrhizae. Boca Raton: CRC Press, 132-146.

Bakken, L. R., Lindahl, V. 1995. Recovery of bacteria cells from soil. In: Trevors, J. T., van Elsas, J. D. eds. Nucleic acids in the environment. New York: Springer-Verlag, 9-27.

Bansal, M., Mukerji, K. G. 1994. Positive correlation between VAM-induced changes in root exudation and mycorrhizosphere mycoflora. Mycorrhiza 5: 39-44.

Caron, M. 1989. Potential use of mycorrhizae in control of soil-borne diseases. Can. J. Plant Pathol. 11: 177-179.

Caron, M., Fortin, J. A., Richard, C. 1986. Effect of phosphorus concentration and *Glomus intraradices* on Fusarium crown and root rot of tomatoes. Phytopathology 76: 942-946.

Catska, V. 1994. Interrelationships between vesicular-arbuscular mycorrhiza and rhizosphere microflora in apple replant disease. Biol. Plant. (Prague) 36: 99-104.

Citernesi, A. S., Fortuna, P., Filippi, C., Bagnoli, G., Giovannetti, M. 1996. The occurrence of antagonistic bacteria in *Glomus mosseae* pot cultures. Agronomie (Paris) 16: 671-677.

Cook, R. J. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. Annu. Rev. Phytopathol. 31: 53-80.

Curl, E. A., Truelove, B. 1986. The rhizosphere. Giessen: Springer-Verlag.

Davies, F. T., Potter, J. R., Linderman, R. G. 1992. Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of pepper plants independant of plant size and nutrient content. J. Plant Physiol. 139: 289-294.

Dehne, H. W. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72: 1115-1119.

Dewey, F. M., Thornton, C. R., Gilligan, C. A. 1997. Use of monoclonal antibodies to detect, quantify and visualize fungi in soils. Adv. Bot. Res. 24: 275-308.

Garcia-Garrido, J. M., Ocampo, J. A. 1988. Interaction between *Glomus mosseae* and *Erwinia carotovora* and its effects on the growth of tomato plants. New phytol. 110: 551-555.

Garcia-Garrido, J. M., Ocampo, J. A. 1989. Effect of VA mycorrhizal infection of tomato on damage caused by *Pseudomonas syringae*. Soil. Biol. Biochem. 21: 165-167.

Giovannetti, M., Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84: 489-500.

Gitaitis, R. D., Beaver, R. W., Voloudakis, A. E. 1991. Detection of *Clavibacter michiganensis* subsp. *michiganensis* in symptomless tomato transplants. Plant Dis. 75: 834-838.

Gleason, M. L., Braun, E. J., Carlton, W. M., Peterson, R. H. 1991. Survival and dissemination of *Clavibacter michiganensis* subsp. *michiganensis* in tomatoes. Phytopathology 81: 1519-1523.

Gleason, M. L., Gitaitis, R. D., Ricker, M. D. 1993. Recent progress in understanding and controlling bacterial canker of tomato in eastern north America. Plant Dis. 77: 1069-1076.

Hewitt, E. 1966. Sand and water culture methods used in the study of plant nutrition. East Malling: Commonwealth Agricultural Bureaux.

Hooker, J. E., Black, K. E. 1995. Arbuscular mycorrhizal fungi as components of sustainable soil-plant systems. Crit. Rev. Biotechnol. 15: 201-212.

Hooker, J. E., Jaizme-Vega, M., Atkinson, D. 1994. Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In: Gianinazzi, S., Schüepp, S. G., eds. Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Basel: Birkhäuser Verlag, 191-200.

Hwang, S. F., Chang, K. F., Chakravarty, P. 1992. Effects of vesicular-arbuscular mycorrhizal fungi on the development of Verticillium and Fusarium wilts of alfalfa. Plant Dis. 76: 239-243.

Jalali, B., Jalali, I. 1991. Mycorrhiza in plant disease control. In: Dilip, K., Arora, B. R., Mukerji, K. G., Knudsen, G. R., eds. Hanbook of applied mycology, vol. 1. New York: Marcel Dekker Inc., 131-153.

Joner, E. J., Jakobsen, I. 1995. Growth and extracellular phosphatase activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter. Soil Biol. Biochem. 27: 1153-1159.

Jones, J. B., Jones, J. P., Stall, R. E., Zitter, T. A. 1991. Compendium of tomato disease. St-Paul, Minnesota: APS Press.

Kabir, Z., Ohalloran, I. P., Hamel, C. 1997. Overwinter survival of arbuscular mycorrhizal hyphae is favored by attachment to roots but diminished by disturbance. Mycorrhiza 7: 197-200.

Kaye, J. W., Pfleger, F. L., Stewart, E. L. 1983. Interaction of *Glomus fasciculatum* and *Pythium ultimum* on greenhouse-grown poinsettia. Can. J. Bot. 62: 1575-1579.

Kormanik, P. P., McGraw, A. C. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. In: Schenck, N. C., eds. Methods and principles of mycorrhizal research. St-Paul, Minnesota: APS Press, 37-45.

Krishna, K. R., Bagyaraj, D. J. 1982. Interaction between *Glomus fasciculatum* and *Sclerotium rolfsii* in peanut. Can. J. Bot. 61: 2349-2351.

Larsen, J., Jakobsen, I. 1996. Interactions between a mycophagous collembola, dry yeast and the external mycelium of an arbuscular mycorrhizal fungus. Mycorrhiza 6: 259-264.

Lehmann, E. 1975. Nonparametrics-statistical methods based on ranks. Oakland, CA: Holden-Day.

Linderman, R. G. 1988. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. Phytopathology 78: 366-370.

Martin, J. K. 1975. Comparison of agar media for counts of viable soil bacteria. Soil Biol. Biochem. 7: 401-402.

McAllister, C. B., Garcia-Romera, I., Godeas, A., Ocampo, J. A. 1994. Interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae* - effects on plant growth, arbuscular mycorrhizas and the saprophyte inoculants. Soil Biol. Biochem. 26: 1363-1367.

Meyer, J. R., Linderman, R. G. 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil. Biol. Biochem. 18: 191-196.

Nehl, D. B., Allen, S. J., Brown, J. F. 1997. Deleterious rhizosphere bacteria - an integrating perspective. Appl. Soil Ecol. 5: 1-20.

Nelsen, C. E., Safir, G. R. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta (Heidelb.) 154: 407-413.

Otten, W., Gilligan, C. A., Thornton, C. R. 1997. Quantification of fungal antigens in soil with a monoclonal antibody-based ELISA - analysis and reduction of soil-specific bias. Phytopathology 87: 730-736.

Papavizas, G. C., Lumsden, R. D. 1980. Biological control of soilborne fungal propagules. Ann. Rev. Phytopathol. 18: 389-413.

Paulitz, T. C., Linderman, R. G. 1991. Mycorrhizal interactions with soil organisms. In: Dilip, K., Arora, B. R., Mukerij, K. G., Knudsen, G. R., eds. Handbook of applied mycology, vol. 1. New York: Marcel Dekker Inc., 77-129.

Redecker, D., Thierfelder, H., Werner, D. 1995. A new cultivation system for arbuscular mycorrhizal fungi on glass beads. Angew. Bot. 69: 189-191.

Rovira, A. D., Newman, E. I., Bowen, H. J., Campbell, R. 1974. Quantitative assessment of the rhizoplane microflora by direct microscopy. Soil Biol. Biochem. 6: 211-216.

SAS Institute Inc. 1996. SAS Institute Inc., Cary, NC.

Schloter, M., Assmus, B., Hartmann, A. 1995. The use of immunological methods to detect and identify bacteria in the environment. Biotechnol. Adv. 13: 75-90.

Schloter, M., Bode, W., Hartmann, A., Beese, F. 1992. Sensitive chemoluminescence-based immunological quantification of bacteria in soil extracts with monoclonal antibodies. Soil. Biol. Biochem. 34: 399-403.

Schreiner, R. P., Bethlenfalvay, J. 1995. Mycorrhizal interactions in sustainable agriculture. Crit. Rev. Biotechnol. 15: 271-285.

Schwab, S. M., Leonard, R. T., Menge, J. A. 1984. Quantitative and qualitative comparison of root exsudates of mycorrhizal and nonmycorrhizal plant species. Can. J. Bot. 62: 1227-1231.

Secilia, J., Bagyaraj, D. J. 1987. Bacteria and actinomycetes associated with pot cultures of vesicular-arbuscular mycorrhizas. Can. J. Microbiol. 33: 1069-1073.

Shirakawa, T., Sasaki, T. 1988. A selective medium for isolation of *Corynebacterium michiganense* pv. *michiganense*, the pathogen of tomato bacterial canker disease. Ann. Phytopathol. Soc. Jpn. 54: 540-543.

Simon, A., Rovira, A. D., Sands, D. C. 1973. An improved selective medium for isolating fluorescent Pseudomonads. J. Appl. Bacteriol. 36: 141-145.

Singh, R. P., Singh, U. S. 1995. Molecular methods in plant pathology. Boca Raton: CRC Press.

Smith, S. E., Gianinazzi-Pearson, V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39: 221-244.

Smith, S. E., Read, D. J. 1997. Mycorrhizal symbiosis. Cambridge: Academic Press.

St-Arnaud, M., Hamel, C., Caron, M., Fortin, J. A. 1994. Inhibition of *Pythium ultimum* in roots and growth substrate of mycorrhizal *Tagetes patula* colonized *with Glomus intraradices*. Can. J. Plant Pathol. 16: 187-194.

St-Arnaud, M., Hamel, C., Caron, M., Fortin, J. A. 1995. Endomycorhizes VA et sensibilité des plantes aux maladies: synthèse de la littérature et mécanismes d'interaction potentiels. In: Fortin, J. A., Charest, C., Piché, Y., eds. La symbiose mycorhizienne - état des connaissances. Frelighsburg: Orbis Publishing, 51-87.

St-Arnaud, M., Hamel, C., Vimard, B., Caron, M., Fortin, J. A. 1997. Inhibition of *Fusarium oxysporum* f. sp. *dianthi* in the non-VAM species *Dianthus caryophyllus* by co-culture with *Tagetes patula* companion plants colonized by *Glomus intraradices*. Can. J. Bot. 75: 998-1005.

Sutton, J. C., Sheppard, B. R. 1976. Aggregation of sand-dune by endomycorrhizal fungi. Can. J. Bot. 54: 326-333.

Sutula, C. L., Gillett, J. M., Morrissey, S. M., Ramsdell, D. C. 1986. Interpreting ELISA data and establishing the positive-negative threshold. Plant Dis. 70: 722-726.

Thornton, C. R., Dewey, F. M., Galligan, C. A. 1993. Development of monoclonal antibody-based immunological assays for the detection of live propagules of *Rhizoctonia solani* in soil. Plant Pathol. (Oxf.) 42: 763-773.

Tisdall, J. M. 1994. Possible role of soil microorganisms in aggregation in soils. Plant Soil 159: 115-121.

Wacker, T. L., Safir, G. R., Stephens, C. T. 1990. Effect of *Glomus fasciculatum* on the growth of asparagus and the incidence of Fusarium root rot. J. Am. Soc. Hortic. Sci. 115: 550-554.

Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Ann. Rev. Phytopathol. 26: 379-407.

Whipps, J. M. 1997. Developments in the biological control of soil-borne plant pathogens. Adv. Bot. Res. 26: 1-134.

Wright, S. F., Upadhyaya, A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161: 575-586.

TABLES

Table 1. Effect of mycorrhizal treatment on % of root length colonized by *Glomus intraradices* (AM) and shoot dry weight.

Treatment	AM (%)[a] Mean ± SD	Shoot dry weight (g) Mean ± SD
Myc+	36.50 a ± 6.83[b]	0.110 a ± 0.03
Myc-	0.00 b ± 0.00	0.098 a ± 0.03

[a]Percent root length bearing fungal structures of *G. intraradices,* as evaluated with the gridline intersect method (Giovannetti & Mosse 1980).

[b]Means of 24 replicates ± standard deviation. Within each column, means with different letters are significantly different ($P<0.05$) by Tukey's studentized range tests.

Table 2. Effect of mycorrhizal and compartmental treatments on hyphal presence, root length and soil pH.

Treatment	Compartment	Hyphal presence (%/cm^2)[a]	Root length (cm)	soil pH
Myc+	Bulk soil compartment	3.25 ± 1.50 a[b]	0.0 ± 0.00 a	6.85 ± 0.07 a
	Root compartment	10.75 ± 1.71 b	3.9 ± 4.48 b	6.89 ± 0.05 a
	Hyphal compartment	11.00 ± 1.15 b	0.0 ± 0.00 a	6.88 ± 0.06 a
Myc-	Bulk soil compartment	1.50 ± 1.00 a[c]	0.0 ± 0.00 a	6.82 ± 0.09 a
	Root compartment	3.50 ± 3.87 a	5.9 ± 7.21 b	6.88 ± 0.05 a
	Hyphal compartment	3.75 ± 4.11 a	0.0 ± 0.00 a	6.86 ± 0.07 a

[a]Percent of AM fungal hyphae presence on 1 cm^2 after soil extraction.

[b] Means of four replicates ± standard deviation. Within each column (for myc+ treatments), means with different letters are significantly different ($P<0.05$) by Tukey's studentized range tests.

[c] Means of four replicates ± standard deviation. Within each column (for myc- treatments), means with different letters are significantly different ($P<0.05$) by Tukey's studentized range tests.

FIGURES

LEGEND OF FIGURES

Figure 1. Compartmentalized growth unit system with a 5 week old tomato plant. **A,** top view **B,** side view.

Figure 2. Relationship between the concentration of suspensions of a pure culture of *Clavibacter michiganensis* subsp. *michiganensis* and ELISA absorbance for identical series divided over three absorbance readings and analyzed on the same day. Bacterial number is based on mean of triplicates plated on Nutrient Agar.

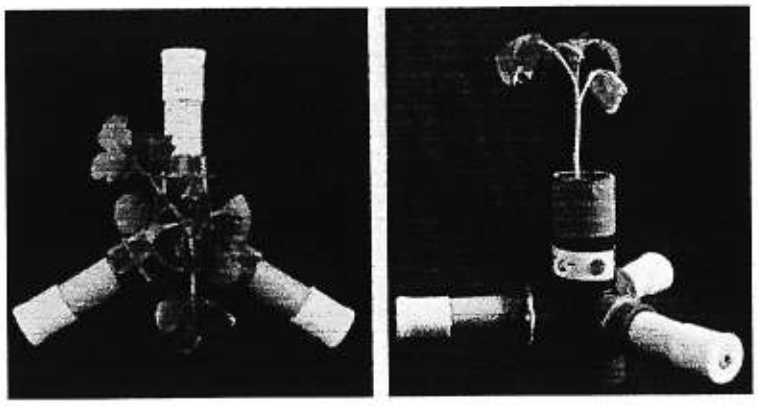
Figure 3. Effect of soil compartmentation on *Clavibacter michiganensis* subsp. *michiganensis* density evaluated by ELISA absorbance. Values are mean of 12 replicates. Error bars = standard deviation. Within each compartment, means with different letters are significantly different ($P<0.05$) by Tukey's studentized range tests.

Figure 4. Effect of soil compartmentation on total bacterial number. Values are mean of four replicates. Columns with a different letter are significantly different by a Tukey's studentized range test ($P<0.05$).

Figure 5. Effect of soil compartmentation on Gram- bacterial number. Values are mean of four replicates. Columns with a different letter are significantly different by a Tukey's studentized range test ($P<0.05$).

Figure 6. Effect of soil compartmentation on total fluorescent Pseudomonads number. Values are mean of four replicates. Within each compartment, columns topped with an asterisk (*) indicate a significant difference by a Tukey's studentized range test ($P<0.05$). In myc+ system (a,b,c) or myc- system (x,y,z), columns with a different letter are significantly different by a Tukey's studentized range test ($P<0.05$).

Figure 1.



A. **B.**

Figure 2.

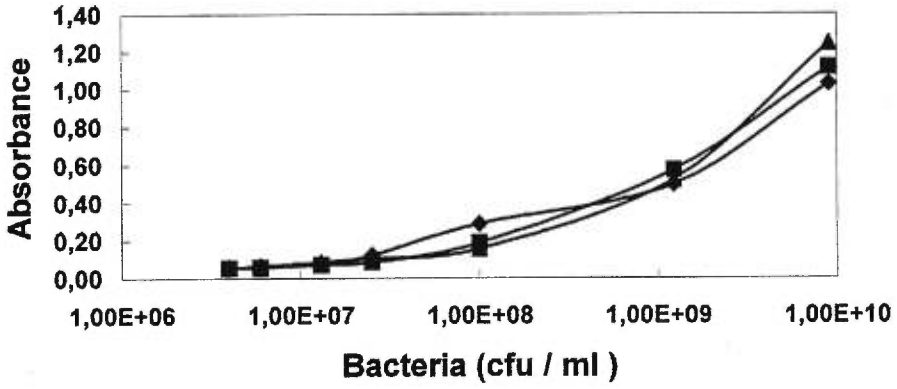

1,40
1,20
1,00
0,80
0,60
0,40
0,20
0,00
Absorbance
1,00E+06
1,00E+07
1,00E+08
1,00E+09
1,00E+10
Bacteria (cfu / ml)

Figure 3.

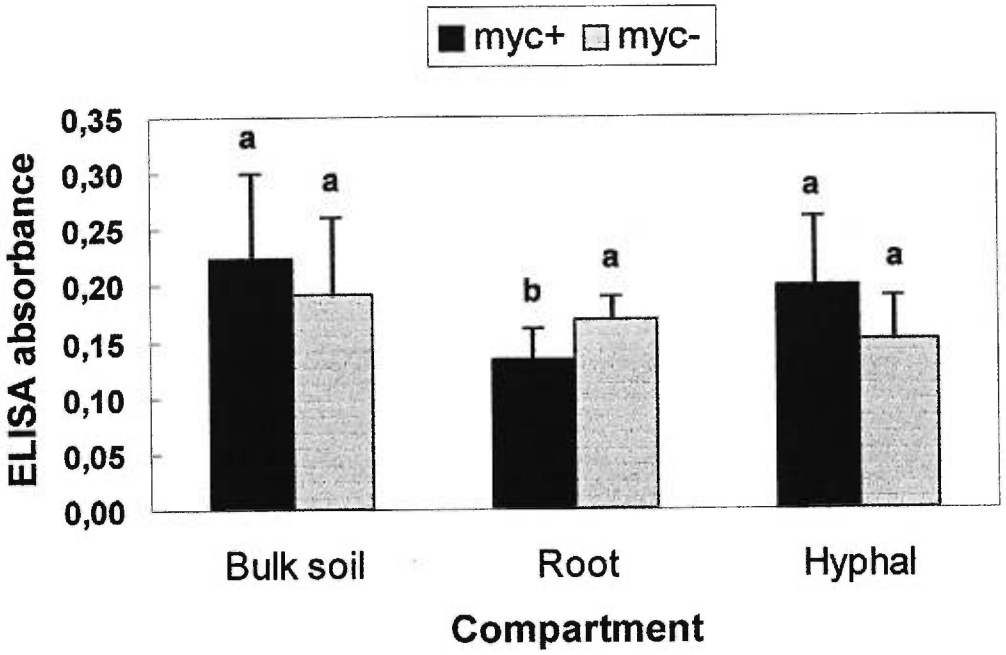

myc+ myc-
ELISA absorbance
0,35
0,30
0,25
0,20
0,15
0,10
0,05
0,00
a
a
b
a
a
a
Bulk soil
Root
Hyphal
Compartment

Figure 4.

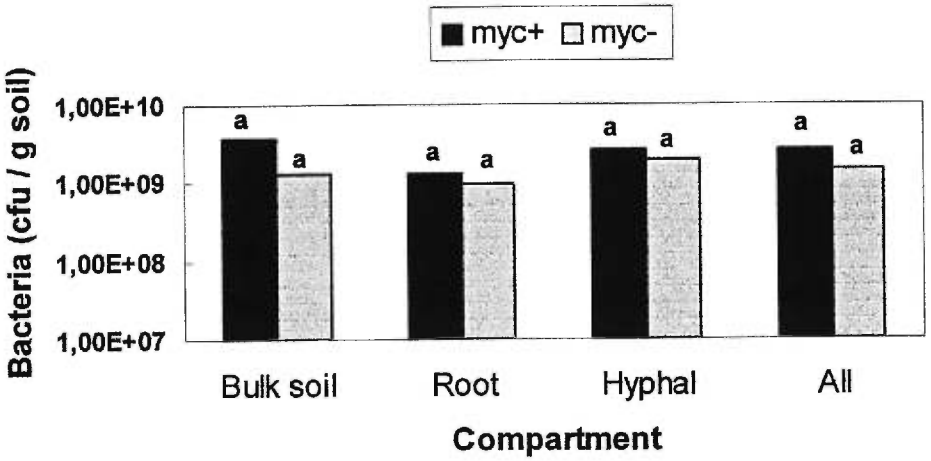

myc+ myc-
Bacteria (cfu / g soil)
1,00E+10
1,00E+09
1,00E+08
1,00E+07
a
a
a
a
a
a
a
a
Bulk soil
Root
Hyphal
All
Compartment

Figure 5.

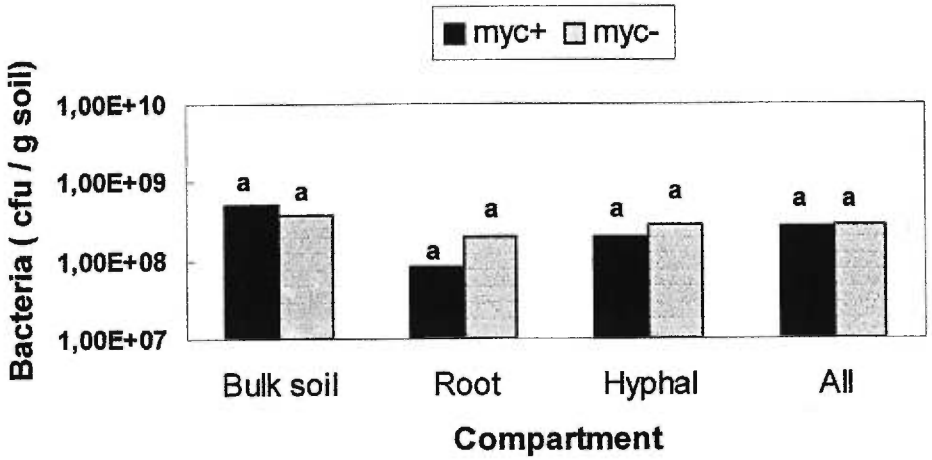
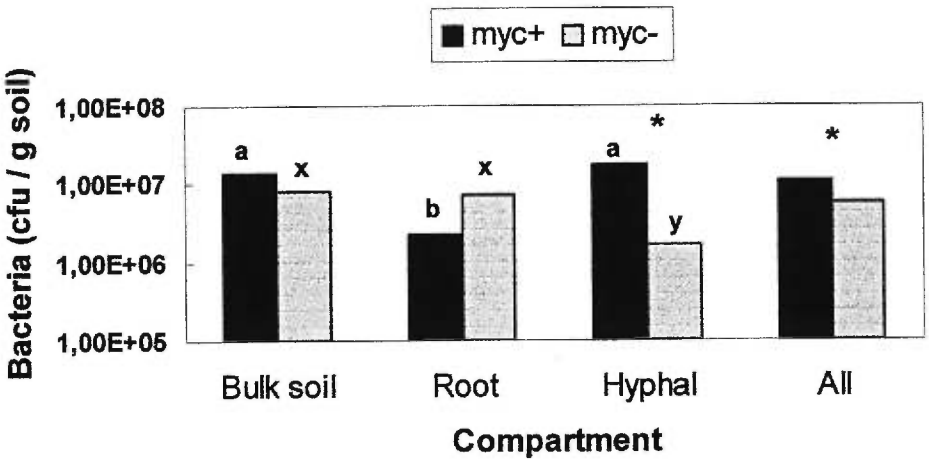

myc+ myc-
Bacteria (cfu / g soil)
1,00E+10
1,00E+09
1,00E+08
1,00E+07
a a
a a
a a
a a
Bulk soil
Root
Hyphal
All
Compartment

Figure 6.


myc+ myc-
Bacteria (cfu / g soil)
1,00E+08
1,00E+07
1,00E+06
1,00E+05
a
x
b
x
a
*
y
*
Bulk soil
Root
Hyphal
All
Compartment

4. DISCUSSION GÉNÉRALE

Dans un premier temps, il a été démontré que le *Glomus intraradices* libère des substances via sa phase extramatricielle et que ces substances ont un effet différentiel sur la croissance de plusieurs micro-organismes du sol. Dans un second temps, il a été démontré, à l'aide d'outils classiques et l'adaptation d'outils moléculaires, que le *G. intraradices* réduit la présence du *Clavibacter michiganensis* subsp. *michiganensis* dans la mycorhizosphère de tomates et influence également la croissance d'un autre groupe bactérien d'importance écologique.

4.1 Effets des substances libérées par le *Glomus intraradices*

Il s'agissait d'isoler toutes les substances solubles libérées dans le compartiment distal ne contenant que la phase extramatricielle du *G. intraradices* d'une culture *in vitro* sur racines de carottes transformées. Cet extrait brut de substances fut concentré par lyophilisation et diverses concentrations furent évaluées pour leur effet sur la croissance de différents micro-organismes particulièrement représentatifs de la mycorhizosphère. Les témoins consistèrent en extraits provenant de compartiments ne contenant pas le champignon MA. Les substances isolées engendrèrent diverses réponses de croissance en fonction des micro-organismes étudiés. De plus, les réponses observées furent généralement corrélées aux concentrations des extraits. La germination conidienne du *Fusarium oxysporum* f. sp. *chrysanthemi*, un champignon parasite, fut significativement réduite en présence des extraits provenant de cultures de *G. intraradices*. La germination conidienne du *Trichoderma harzianum*, un champignon saprophyte, fut par contre

significativement stimulée en présence des mêmes extraits. La croissance bactérienne du *Pseudomonas chlororaphis*, une bactérie de type PGPR (plant growth promoting rhizobacteria), fut également significativement stimulée et celle du *Clavibacter michiganensis* subsp. *michiganensis*, une bactérie pathogène, non-affectée. L'effet d'une modification de pH, facteur important pouvant influencer les résultats, fut éliminée comme facteur explicatif des résultats compte tenu des différentes mesures effectuées. Ainsi, la libération de substances dans le milieu de culture du *G. intraradices* fut retenue comme hypothèse probable pouvant expliquer les résultats obtenus. Néanmoins, à l'aide du protocole utilisé, nous n'avons pas tenté de déterminer la nature et la composition des substances en cause. Ainsi, ces substances peuvent être activement libérées dans l'environnement par la phase extramatricielle du *G. intraradices* ou être de nature intra-hyphale. Dans les deux cas, ces substances se retrouvent probablement dans la mycorhizosphère dû à la décomposition naturelle et fréquente des hyphes et la rupture du réseau mycélien par différents micro-organismes du sol, causant un flux du matériel intra-hyphal dans le sol (Fitter & Garbaye 1994; Klironomos & Ursic 1998; Larsen & Jakobsen 1996; Moore *et al.* 1985; Reddell *et al.* 1997; Thimm & Larink 1995).

Il a souvent été proposé que les champignons MA étaient en mesure de produire des substances pouvant interférer directement avec des micro-organismes. Cette hypothèse n'avait toutefois été démontrée que de façon circonstancielle (Andrade *et al.* 1997; Linderman 1988; Paulitz & Linderman 1991; St-Arnaud *et al.* 1995). À notre connaissance, il s'agit de la première démonstration claire qu'un extrait brut de milieu de culture ayant servi à la culture d'un champignon MA comporte des substances pouvant directement influencer la croissance de différents micro-organismes. Ces résultats suggèrent fortement que le mycélium du *G. intraradices* relâche des

susbstances dans l'environnement et que ces substances ont un impact important sur l'équilibre microbien du sol. Les substances impliquées peuvent ainsi avoir un effet direct sur la réduction de populations de parasites en sol ou un effet indirect en stimulant la croissance d'autres micro-organismes, qui eux, peuvent à leur tour interagir avec des parasites par des mécanismes tels l'antibiose, la compétition pour les ressources ou le parasitisme.

Le volume de sol occupé par la phase extramatricielle de champignons MA est de beaucoup supérieur à celui exploré par les racines (Smith & Read 1997). Ainsi, le mycélium de champignons MA a le potentiel d'agir comme première ligne de défense de plantes endomycorhizées en modifiant favorablement l'équilible microbien de la mycosphère.

4.2 Développement d'outils quantitatifs afin d'investiguer la mycorhizosphère

En marge de la compréhension fondamentale des mécanismes impliqués en conditions *in vitro*, il est essentiel de vérifier si de tels mécanismes opèrent toujours en conditions naturelles. Cette procédure nécessite le développement d'outils permettant de mettre en lumière des effets biologiques subtils en présence d'une multitude d'interactions inhérentes au milieu naturel. Pour nos besoins, un système de culture de plantes entières en sol, permettant de bien délimiter les différentes zones importantes de la mycorhizosphère, fut développé. Ce système a permis de séparer l'influence des racines d'une plante de celle des hyphes d'un champignon MA sur la dynamique de croissance de différents micro-organismes du sol. Par le passé, les études portant sur la dynamique de population en sol utilisèrent des techniques de quantification basées sur la

dilution et le dénombrement sur milieu semi- ou sélectifs ou encore de comptes totaux en microscopie. À notre connaissance, aucune étude utilisant des outils de quantification moléculaires n'avait été effectuée dans le domaine des interactions de la mycorhizosphère.

L'émergence récente de techniques moléculaires, telles la technologie de la Polymerase Chain Reaction (PCR) ou de l'Enzyme Link ImmunoSorbent Assay (ELISA), avec approche quantitative, permettent maintenant de suivre des populations de micro-organismes spécifiques sous différentes conditions (Singh & Singh 1995). Dans le cas du PCR, on quantifie à l'aide d'amorces spécifiques une réaction exponentielle d'amplification d'un brin d'ADN visé. Par l'ajout d'un autre brin, de longueur différente mais amplifié par les mêmes amorces, il devient possible de quantifier un ratio d'amplification et ainsi une quantité d'ADN de départ (Innis *et al.* 1990). L'ELISA nécessite un anticorps polyclonal ou monoclonal le plus spécifique possible et dirigé contre un déterminant antigénique de surface d'un micro-organisme visé. La réaction antigène-anticorps, attachée à une phase solide, généralement en plaque multipuits, peut par la suite être quantifiée par spectrophotométrie (Crowther 1995).

Très peu d'études ont appliqué ce potentiel de quantification en conditions de sol (Dewey *et al.* 1997; Schloter *et al.* 1995; Schneegurt & Kulpa 1998). Ceci est dû notamment à la difficulté d'isoler adéquatement certains groupes de micro-organismes édaphiques et également à l'interférence de différentes substances du sol avec les composés chimiques utilisés dans ces protocoles (Akkermans *et al.* 1995; Trevors & Van Elsas 1995). La présence de certaines substances, telles les acides humiques, semble représenter le principal problème (Jackson *et al.* 1997; Weaver *et al.* 1994). Néanmoins, différents protocoles d'extraction de micro-organismes du sol ont récemment été mis au

point (Akkermans *et al.* 1995; Lindahl & Bakken 1995; Picard *et al.* 1992; Trevors & Van Elsas 1995). Ainsi, en combinant des protocoles d'extraction adéquats à une technologie de détection-quantification très précise, il devient possible de suivre la dynamique de populations d'un micro-organisme en sol dans la mycorhizosphère.

Pour nos besoins, l'ELISA apparut l'outil de choix, d'autant plus qu'il existe des anticorps dirigés contre divers micro-organismes produits et vendus dans le commerce. La technique du Double Antibody Sandwich (DAS)-ELISA semble constituer présentement l'approche conférant le meilleur potentiel de quantification à l'aide de la technologie de l'ELISA en substrat complexe (Crowther 1995; Singh & Singh 1995). Cette technique repose sur une capture du déterminant antigénique visé entre deux anticorps, réduisant ainsi les réactions croisées avec d'autres déterminants antigéniques similaires. En sol, il apparaît crucial d'obtenir la meilleure spécificité possible compte tenu des millions de micro-organismes présents. Il fut décidé d'utiliser un anticorps polyclonal acheté dans le commerce (Agdia Inc., Elkhart, IN, USA) et dirigé contre le *Clavibater michiganensis* subsp. *michiganensis*, une bactérie pathogène causant le chancre bactérien de la tomate. La limite de détection généralement obtenu avec des anticorps polyclonaux est généralement de l'ordre de 10^6 (Schloter *et al.* 1995), ce qui ne constitue pas un problème dans le cadre de ce type d'étude. En effet, on raporte des populations très élevées dans les productions de tomates infectées avec le *C. m. michiganensis*, condition à reproduire dans nos protocoles (Gleason *et al.* 1991). En marge de la démonstration *in vitro* d'interaction directe entre un champignon MA et différents micro-organismes, nous voulions maintenant vérifier la présence d'interactions directes ou indirectes en sol dans la mycorhizosphère.

4.3 Interactions entre le *Glomus intraradices* et le *C. m. michiganensis* en sol

Un nouveau système expérimental fut développé afin de séparer physiquement les principales régions de la mycorhizosphère à l'aide de membranes de différentes porosités. Ainsi, le système comporta trois compartiments simulant respectivement la rhizosphère, la mycosphère et le sol distant. L'étude visa à quantifier la présence du *C. m. michiganensis* dans les principales zones d'importance de la mycorhizosphère de tomates inoculées depuis un temps variable (12 h, 36 h, 60 h) avec ce parasite en présence ou absence du champignon MA *G. intraradices*. Il fut démontré que le facteur temps ne donnait pas de résultats significativement différents et l'analyse porta ainsi sur l'ensemble des résultats des trois durées d'incubation évalués conjointement. Une limite de détection de 4×10^6 bactéries · ml^{-1} en culture pure et de 6×10^6 bactéries · ml^{-1} en sol fut obtenue. Les résultats démontrèrent que la présence de racines mycorhizées réduit significativement la présence du *C. m. michiganensis* en comparaison à des racines non-mycorhizées. Par contre, la présence seule du *G. intraradices* n'eut pas d'impact significatif sur la présence du *C. m. michiganensis*. Ainsi, en concordance avec les résultats obtenus dans le cadre du premier article, on démontra de nouveau l'incapacité du *G. intraradices* à influencer directement et significativement la croissance du *C. m. michiganensis*. Néanmoins, le *G. intraradices* possède la capacité d'influencer indirectement la croissance du *C. m. michiganensis*. Ceci, pour les conditions étudiées, semble se produire via une modification des exsudats de racines mycorhizées par rapport aux racines témoins non-mycorhizées. Il est clairement démontré que les exsudats de plantes mycorhizées sont différents de ceux provenant de plantes non-mycorhizées (Bansal & Mukerji 1994; Schwab *et al.* 1984). Ainsi, nous démontrons une relation

possible entre la modification d'exsudation et une réduction de populations de pathogènes.

Des études parallèles, par décompte sur milieux sélectifs, ont démontré que le *G. intraradices* stimule significativement la présence des pseudomonas fluorescents sous les conditions étudiées. Ces micro-organismes sont connus pour leur effet généralement bénéfique sur la réduction de populations de parasites et également sur la croissance des plantes (Nehl *et al.* 1997; Weller 1988). Aucune corrélation n'a toutefois pu être obtenue entre la réduction des populations du *C. m. michiganensis* et l'augmentation des pseudomonas fluorescents. D'autres décomptes sur milieux sélectifs furent effectués afin de quantifier respectivement les bactéries totales et les bactéries Gram-. Les résultats démontrèrent que le *G. intraradices* n'influence pas de façon significative la dynamique quantitative de population de ces groupes de micro-organismes dans les différentes zones de la mycorhizosphère sous les conditions étudiées. De plus, différentes mesures physiques et chimiques de paramètres du système expérimental furent évaluées. Les données obtenues ne démontrèrent aucune modification du pH du sol ou du poids sec des parties aériennes des plants de tomates. Ainsi, la modification d'exsudation demeure l'explication la plus plausible afin d'expliquer les résultats obtenus.

4.4 Conclusion

Le *G. intraradices* possède la capacité d'interagir directement et indirectement avec des micro-organismes de la mycorhizosphère. De façon générale, il a démontré une tendance très nette à réduire la croissance de parasites et stimuler la croissance de micro-organismes bénéfiques pour la plante. Ainsi, sous les conditions étudiées, l'équilibre

microbien de la mycorhizosphère abritant le *G. intraradices* est modifé et semble généralement plus bénéfique en ce qui à trait à la protection de la plante contre différents parasites. Le champignon MA, par sa multiplicité d'action, exerce un effet marqué sur la mycorhizosphère et tend à améliorer les conditions phytosanitaires présentes. Ainsi, le *G. intraradices* possède un excellent potentiel en tant qu'agent de biocontrôle. Non seulement ses capacités d'antagoniste sont présentes en conditions *in vitro*, mais également en sol. De par son statut de symbiote obligatoire, cet organisme possède un fort potentiel à persister dans l'environnement, autre qualité essentielle à tout agent de lutte biologique. De plus, sa présence stimule la croissance d'autres micro-organismes connus pour leur pouvoir antagoniste face à différents parasites et bénéfiques pour la plante. Cette situation ouvre un créneau commercial pour la production d'inoculums mycorhiziens mixtes de concert avec d'autres micro-organismes, dans une perspective de lutte biologique et/ou intégrée. Néanmoins, plusieurs études devront être menées afin de mieux cerner encore l'influence du *G. intraradices* et autres champignons MA dans la mycorhizosphère. Une meilleure compréhension des interactions complexes de la mycorhizosphère va certainement constituer un défi de taille pour les années à venir. Ces études vont toutefois tendre à permettre le développement d'approches utiles en lutte biologique et/ou intégrée, beaucoup moins dommageables pour l'environnement que l'utilisation massive de pesticides.

5. BIBLIOGRAPHIE GÉNÉRALE

Agrios, G. N. 1997. Plant Pathology. Fourth ed. San Diego: Academic Press.

Akkermans, A. D. L., Van Elsas, J. D., De Bruijn, F. J. 1995. Molecular microbial ecology manual. Dordrecht: Kluwer Academic Publishers.

Aldwell, R. E. B., Hall, I. R., Smith, J. M. B. 1985. Enzyme-linked immunosorbent assay as an aid to taxonomy of the Endogonaceae. Trans. Br. Mycol. Soc. 84:399-402.

Allen, R. N., Cunningham, G. L. 1983. Effects of vesicular-arbuscular mycorrhizal fungi of *Distichlis sepicata* under three salinity levels. New Phytol. 96:227-236.

Andrade, G., Mihara, K. L., Linderman, R. G., Bethlenfalvay, G. J. 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192:71-79.

Azcón-Aguilar, C., Barea, J. M. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens - an overview of the mechanisms involved. Mycorrhiza 6:457-464.

Bago, B., Azcón-Aguilar, C. 1997. Changes in the rhizospheric pH induced by arbuscular mycorrhiza formation in onion (Allium cepa L). Z. Pflanzenernahr. Bodenkd. 160:333-339.

Bagyaraj, J. 1984. Biological interactions with VA mycorrhizal fungi. In: Conway, L. I., Powell, C. L., Bagyaraj, J., eds. VA mycorrhizae. Boca Raton: CRC Press, 132-146.

Bansal, M., Mukerji, K. G. 1994. Positive correlation between VAM-induced changes in root exudation and mycorrhizosphere mycoflora. Mycorrhiza 5:39-44.

Benhamou, N., Fortin, J. A., Hamel, C., St-Arnaud, M., Shatilla, A. 1994. Resistance responses of mycorrhizal Ri T-DNA-transformed carrot roots to infection by *Fusarium oxysporum* f. sp. *chrysanthemi*. Phytopathology 84:958-968.

Bonfante, P., Perotto, S. 1995. Strategies of arbuscular mycorrhizal fungi when infecting host plants. New Phytol. 130:3-21.

Bonfante-Fasolo, P., Vian, B., Testa, B. 1986. Ultrastructural localization of chitin in the cell wall of a fungal spore. Biol. Cell 57:265-270.

Caron, M. 1989. Potential use of mycorrhizae in control of soil-borne diseases. Can. J. Plant Pathol. 11:177-179.

Caron, M., Fortin, J. A., Richard, C. 1986. Effect of phosphorus concentration and *Glomus intraradices* on Fusarium crown and root rot of tomatoes. Phytopathology 76:942-946.

Catska, V. 1994. Interrelationships between vesicular-arbuscular mycorrhiza and rhizosphere microflora in apple replant disease. Biol. Plant. 36:99-104.

Citernesi, A. S., Fortuna, P., Filippi, C., Bagnoli, G., Giovannetti, M. 1996. The occurrence of antagonistic bacteria in *Glomus mosseae* pot cultures. Agronomie 16:671-677.

Crowther, J. R. 1995. ELISA Theory and practice Vol. 42. Totowa: Humana Press.

Dalpé, Y. 1995. Systématique des endomycorhizes à arbuscules: de la myco-paléontologie à la biochimie. In: Fortin, J. A., Charest, C., Piché, Y, eds. La symbiose mycorhizienne - état des connaissances. Frelighsburg: Orbis Publishing, 1-20.

Davies, F. T., Potter, J. R., Linderman, R. G. 1992. Mycorrhiza and repeated drought exposure affect drought resistance and extraradical hyphae development of

pepper plants independant of plant size and nutrient content. J. Plant Physiol. 139:289-294.

Dehne, H. W. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72:1115-1119.

Dewey, F. M., Thornton, C. R., Gilligan, C. A. 1997. Use of monoclonal antibodies to detect, quantify and visualize fungi in soils. Adv. Bot. Res. 24:275-308.

Fitter, A. H., Garbaye, J. 1994. Interactions between mycorrhizal fungi and other soil organisms. Plant Soil 159:123-132.

Garcia-Garrido, J. M., Ocampo, J. A. 1988. Interaction between *Glomus mosseae* and *Erwinia carotovora* and its effects on the growth of tomato plants. New Phytol. 110:551-555.

Garcia-Garrido, J. M., Ocampo, J. A. 1989. Effect of VA mycorrhizal infection of tomato on damage caused by *Pseudomonas syringae*. Soil Biol. Biochem. 21:165-167.

Gerdemann, J. W., Trappe, J. M. 1974. The Endogonaceae in the Pacific Northwest. Mycologia Memoir no.5:1-76.

Gerdemann, J. W., Trappe, J. M. 1975. Taxonomy of the Endogonaceae. In: Sanders, F. E., Mosse, B., Tinker, P. B., eds. Endomycorrhizas. London: Academic Press, 35-51.

Gianinazzi-Pearson, V., Dumas-Gaudot, E., Gollotte, A., Tahiri-Alaoui, A., Gianinazzi, S. 1996. Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. New Phytol. 133:45-57.

Gleason, M. L., Braun, E. J., Carlton, W. M., Peterson, R. H. 1991. Survival and dissemination of *Clavibacter michiganensis* subsp. *michiganensis* in tomatoes. Phytopathology 81:1519-1523.

Harley, J. L. 1985. Specificity and penetration of tissues by mycorrhizal fungi. Proc. Indian Acad. Sci. Plant Sci. 94:99-109.

Harrison, M. J., Dixon, R. A. 1993. Isoflavonoid accumulation and expression of defense gene transcripts during the establishment of vesicular-arbuscular mycorrhizal associations in roots of *Medicago truncatula*. Mol. Plant-Microbe interact. 6:643-654.

Hepper, C. M., Sen, R., Azcón-Aguilar, C., Grace, C. 1988. Variation in certain isozymes among different geographical isolates of the vesicular-arbuscular mycorrhizal fungi *Glomus clarum*, *Glomus monosporum* and *Glomus mosseae*. Soil Biol. Biochem. 20:51-59.

Hetrick, B. A. D., Wilson, G. W. T., Cox, T. S. 1993. Mycorrhizal dependance of modern wheat cultivars and ancestors: a synthesis. Can. J. Bot. 71:512-518.

Hooker, J. E., Jaizme-Vega, M., Atkinson, D. 1994. Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In Gianinazzi, S., Schüepp, H., eds. Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Basel: Birkhäuser Verlag, 191-200.

Hwang, S. F., Chang, K. F., Chakravarty, P. 1992. Effects of vesicular-arbuscular mycorrhizal fungi on the development of Verticillium and Fusarium wilts of Alfalfa. Plant Dis. 76:239-243.

Innis, M. A., Gelfand, D. H., Sninsky, J. J., White, T. J., eds. 1990. PCR Protocols: A guide to methods and applications. San Diego: Academic Press.

Jackson, C. R., Harper, J. P., Willoughby, D., Roden, E. E., Churchill, P. F. 1997. A simple, efficient method for the separation of humic substances and DNA from environmental samples. Appl. Environ. Microbiol. 63:4993-4995.

Kaye, J. W., Pfleger, F. L., Stewart, E. L. 1983. Interaction of *Glomus fasciculatum* and *Pythium ultimum* on greenhouse-grown poinsettia. Can. J. Bot. 62:1575-1579.

Klironomos, J. N., Ursic, M. 1998. Density-dependent grazing on the extraradical hyphal network of the arbuscular mycorrhizal fungus, *Glomus intraradices*, by the collembolan, *Folsomia candida*. Biol. Fertil. Soils 26:250-253.

Lambais, M. R., Mehdy, M. C. 1993. Suppression of endochitinase, beta-1-3-endoglucanase, and chalcone isomerase expression in bean vesicular-arbuscular mycorrhizal roots under different soil phosphate conditions. Mol. Plant-Microbe interact. 6:75-83.

Larsen, J., Jakobsen, I. 1996. Effects of a mycophagous collembola on the symbioses between *Trifolium subterraneum* and three arbuscular mycorrhizal fungi. New Phytol. 133:295-302.

Lindahl, V., Bakken, L. R. 1995. Evaluation of methods for extraction of bacteria from soil. FEMS Microbiol. Ecol. 16:135-142.

Linderman, R. G. 1988. Mycorrhizal interactions with the rhizosphere micoflora: the mycorrhizosphere effect. Phytopathology 78:366-370.

McAllister, C. B., Garcia-Romera, I., Godeas, A., Ocampo, J. A. 1994. Interactions between *Trichoderma koningii*, *Fusarium solani* and *Glomus mosseae* - effects on plant growth, arbuscular mycorrhizas and the saprophyte inoculants. Soil Biol. Biochem. 26:1363-1367.

Meyer, J. R., Linderman, R. G. 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Biol. Biochem. 18:191-196.

Moore, J. C., St-John, T. V., Coleman, D. C. 1985. Ingestion of vesicular-arbuscular mycorrhizal hyphae and spores by soil microarthropods. Ecology 66:1979-1981

Morton, J. B. 1988. Taxonomy of VA mycorrhizal fungi: classification nomenclature et identification. Mycotaxon 32:267-324.

Morton, J. B., Benny, G. L. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. Mycotaxon 37:471-491.

Nehl, D. B., Allen, S. J., Brown, J. F. 1997. Deleterious rhizosphere bacteria - an integrating perspective. Applied Soil Ecol. 5:1-20.

Nelsen, C. E., Safir, G. R. 1982. Increased drought tolerance of mycorrhizal onion plants caused by improved phosphorus nutrition. Planta 154:407-413.

Newsham, K. K., Fitter, A. H., Watkinson, A. R. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. J. Ecol. 83:991-1000.

Paulitz, T. C., Linderman, R. G. 1989. Interactions between fluorescent pseudomonads and VA mycorrhizal fungi. New phytol. 113:37-45.

Paulitz, T. C., Linderman, R. G. 1991. Mycorrhizal interactions with soil organisms. In Dilip, B. R., Arora, K., Mukerij, K. G., Knudsen, G. R., eds. Handbook of applied mycology Vol. 1. New York: Marcel Dekker inc., 77-129.

Peterson, R. L., Howarth, M. J., Whittier, D. P. 1981. Interactions between a fungal endophyte and gametophyte cells in *Psilotum nudum*. Can. J. Bot. 59:711-720.

Picard, C., Ponsonnet, C., Paget, E., Nesme, X., Simonet, P. 1992. Detection and enumeration of bacteria in soil by direct DNA extraction and polymerase chain reaction. Appl. and Environ. Microbiol. 58:2717-2722.

Pocock, K., Duckett, J. G. 1984. A comparative ultrastructure analysis of the fungal endophytes in *Cryptothallus mirabilis* Hulm. and other British thalloid hepatics. J Bryol. 13:227-233.

Pocock, K., Duckett, J. G. 1985. On the occurence of branched and swollen rhizoids in British hepatics: their relationship with the substratum and association with fungi. New Phytol. 99:281-304.

Posta, K., Marschner, H., Römheld, V. 1994. Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize. Mycorrhiza 5:119-124.

Reddell, P., Spain, A. V., Hopkins, M. 1997. Dispersal of spores of mycorrhizal fungi in scats of native mammals in tropical forests of northeastern Australia. Biotropica 29:184-192.

Remy, W., Taylor, T. N., Hass, H., Kerp, H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc. Natl. Acad. Sci. USA 91:11841-11843.

Schloter, M., Assmus, B., Hartmann, A. 1995. The use of immunological methods to detect and identify bacteria in the environment. Biotechnol. Adv. 13:75-90.

Schneegurt, M. A., Kulpa, C. F. 1998. The application of molecular techniques in environmental biotechnology for monitoring microbial systems. Biotechnol. Appl. Biochem. 27:73-79.

Schwab, S. M., Leonard, R. T., Menge, J. A. 1984. Quantitative and qualitative comparison of root exsudates of mycorrhizal and nonmycorrhizal plant species. Can. J. Bot. 62:1227-1231.

Simon, L., Bousquet, J., Lévesque, R. C., Lalonde, M. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67-69.

Singh, R. P., Singh, U. S. 1995. Molecular methods in plant pathology. Boca Raton: CRC Press.

Smith, S. E., Gianinazzi-Pearson, V. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39:221-244.

Smith, S. E., Read, D. J. 1997. Mycorrhizal symbiosis 2nd ed. Cambridge: Academic Press.

Spanu, P., Boller, T., Ludwig, A., Wiemken, A., Faccio, A., Bonfante-Fasolo, P. 1989. Chitinase in roots of mycorrhizal *Allium porrum*: regulation and localization. Planta 177:447-455.

Spanu, P., Bonfante-Fasolo, P. 1988. Cell-wall-bound peroxidase activity in roots of mycorrhizal *Allium porrum*. New phytol. 109:119-124.

St-Arnaud, M., Hamel, C., Caron, M., Fortin, J. A. 1994. Inhibition of *Pythium ultimum* in roots and growth substrate of mycorrhizal *Tagetes patula* colonized with *Glomus intraradices*. Can. J. Plant Pathol. 16:187-194.

St-Arnaud, M., Hamel, C., Caron, M., Fortin, J. A. 1995. Endomycorhizes VA et sensibilité des plantes aux maladies: synthèse de la littérature et mécanismes d'interaction potentiels. In: Fortin, J. A., Charest, C., Piché, Y., eds. La symbiose mycorhizienne - état des connaissances. Frelighsburg: Orbis Publishing, 51-87.

Subramanian, K. S., Charest, C., Dwyer, L. M., Hamilton, R. I. 1995. Arbuscular mycorrhizas and water relations in maize under drought stress at tasseling. New Phytol. 129:643-650.

Sutton, J. C., Sheppard, B. R. 1976. Aggregation of sand-dune by endomycorrhizal fungi. Can. J. Bot. 54:326-333.

Taylor, T. N., Remy, W., Hass, H., Kerp, H. 1995. Fossil arbuscular mycorrhizae from the early Devonian. Mycologia 87:560-573.

Thimm, T., Larink, O. 1995. Grazing preferences of some collembola for endomycorrhizal fungi. Biol. Fertil. Soils 19:266-268.

Trappe, J. M. 1987. Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir, G. R., eds. Ecophysiology of VA mycorrhizal plants. Boca Raton: CRC Press, 5-25.

Trappe, J. M., Schenck, N. C. 1982. Taxonomy of fungi forming endomycorrhiza. In Schenck, N. C., eds. Methods and principles of mycological research. St-Paul: American Phytopathological Society, 1-9.

Trevors, J. T., Van Elsas, J. D., eds. 1995. Nucleic acids in the environment. Heidelberg: Springer-Verlag.

Vasanthakrishna, M., Bagyaraj, D. J. 1993. Selection of efficient VA mycorrhizal fungi for inoculating *Casuarina equisetifolia.* Arid Soil Res. Rehabil. 7:377-380.

Vierheilig, H., Alt, M., Mohr, U., Boller, T., Wiemken, A. 1994. Ethylene biosynthesis and activities of chitinase and beta-1,3-glucanase in the roots of host and non-host plants of vesicular-arbuscular mycorrhizal fungi after inoculation with *Glomus mosseae.* J. Plant Physiol. 143:337-343.

Volpin, H., Elkind, Y., Okon, Y., Kapulnik, Y. 1994. A vesicular arbuscular mycorrhizal fungus (*Glomus intraradix*) induces a defense response in alfalfa roots. Plant Physiol. 104:683-689.

Weaver, R. W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., eds. 1994. Methods of soil analysis part 2-microbiological and biochemical properties Vol. 5. Madison: Soil Science Society of America.

Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.

Wright, S. F., Frankesnyder, M., Morton, J. B., Upadhyaya, A. 1996. Time-course study and partial characterization of a protein on hyphae of arbuscular mycorrhizal fungi during active colonization of roots. Plant Soil 181:193-203.

Wright, S. F., Upadhyaya, A. 1996. Extraction of an abundant and unusual protein from soil and comparison with hyphal protein of arbuscular mycorrhizal fungi. Soil Sci. 161:575-586.