VESICULAR-ARBUSCULAR MYCORRHIZAL EFFICIENCY ON APPLE ROOTSTOCKS: EFFECTS OF GENOTYPES AND HERBICIDES

by

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ABSTRACT

M.Sc France Morin Plant Science

VESICULAR ARBUSCULAR MYCORRHIZAL EFFICIENCY

ON APPLE ROOTSTOCKS:

EFFECTS OF GENOTYPES AND HERBICIDES

There has been [illegible] the compatibility of commonly utilized apple rootstocks and VA [illegible] and even less research regarding the effects of herbicides used in orchards on the VAM symbiosis of apple trees. Studies demonstrated that early inoculation of young apple plants, prior to transplanting, results in healthy and vigorous plants with better growth and nutrition than uninoculated plants. We studied the efficiency of VA fungal species and isolates on young apple rootstocks, produced by in vitro propagation. Mycorrhizal inoculation promoted plant growth, dry mass production and leaf P concentration. Mycorrhizal efficiency was associated with larger external hyphal network but showed no relation with the internal colonization. Despite the high P-fertility of the soil used, growth enhancement due to mycorrhizal inoculation was attributed to an improved P nutrition.

In a second experiment, the effect of herbicides currently used in orchards was

tested on the mycorrhizal symbiosis. Paraquat, simazine and dichlobenil were applied to soil with mycorrhizal and non-mycorrhizal apple plants. Mycorrhizae increased herbicide toxicity in apple, as demonstrated by the greatly increased plant mortality. While both paraquat and simazine decreased spore germination in vitro, none of the herbicide treatments affected root colonization in soil. Effects on the photosynthetic rate, measured after herbicide application, indicated a physiological interaction between mycorrhizal colonization and dichlobenil, involved in the toxic response of apple plants.

RÉSUMÉ

M.Sc. France Morin Plant Science

EFFICACITÉ DE CHAMPIGNONS MYCORHIZIENS A VESICULES ET ARBUSCULES SUR DES PORTE-GREFFES DE POMMIERS· EFFETS DU GÉNOTYPE ET DES HERBICIDES.

Il existe peu de recherche sur la compatibilité entre les porte-greffes de pommiers couramment utilisés et les types de champignons à VA, et encore moins concernant les effets des herbicides appliqués en vergers sur la symbiose mycorhizienne des pommiers. Des études ont démontré que l'inoculation précoce des pommiers, avant leur transplantation, procure des plants sains et vigoureux, qui ont une croissance et une nutrition améliorée. Au cours de cette recherche, nous avons étudié l'efficacité de plusieurs espèces et isolats de champignons à vésicules et arbuscules (VA) sur de jeunes porte-greffes de pommiers, issus de la propagation in vitro. L'inoculation mycorhizienne a favorisé la croissance végétale, la production de biomasse et la concentration foliaire en phosphore. L'efficacité mycorhizienne était associée au développement extensif du réseau d'hyphes extraradiculaires mais n'était pas relié au pourcentage de colonization racinaire. Malgré le haut niveau de phosphate du sol, l'augmentation de croissance suite à l'inoculation

mycorhizienne a été attribuée à une meilleure nutrition phosphatée.

Dans une seconde expérience, on a évalué les effets d'herbicides couramment utilisés en vergers, sur la symbiose mycorhizienne. Les herbicides paraquat, simazine et dichlobénil ont été appliqués au sol de plants mycorhizés et non-mycorhizés. La mycorhization a augmenté la phytotoxicité, tel que démontré par la mortalité plus élevée des plants mycorhizés. Alors que le paraquat et la simazine ont tous les deux diminué la germination in vitro des spores, , aucun traitement herbicide n'a affecté la colonisation des racines dans le sol. Les taux de photosynthèse mesurés après application des herbicides ont révélé une interaction physiologique entre la colonisation mycorhizienne et le dichlobénil.

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My codirector, André FORTIN, gave me the determination and motivation to start this degree and involved himself to make it possible Beside his indubitable scientific qualities, I appreciated his dynamism and his kindness. I will always admire his simplicity. I wish to express him my appreciation for the support and confidence he provided me.

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A mes parents,

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Pour le sens des valeurs qu'ils m'ont transmis et pour tout le reste

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Section 1

CONTRIBUTION OF CO-AUTHORS TO MANUSCRIPTS FOR PUBLICATION

The relevant section of the regulation follows:

"Candidates have the option, subject to the approval of their Department, of including, as part of their thesis, copies of the text of a paper(s) submitted for publication, or the clearly-duplicated text of a published paper(s), provided that these copies are bound as an integral part of the thesis.

- If this option is chosen, connecting texts, providing logical bridges between the different papers, are mandatory.
- The thesis must still conform to all other requirements of the "Guidelines Concerning Thesis Preparation" and should be in a literary form that is more than a mere collection of manuscripts published or to be published. The thesis must include, as separate chapters or sections: (1) a Table of Contents, (2) a general abstract in English and French, (3) an introduction which clearly states the rationale and objectives of the study, (4) a comprehensive general review of the background literature to the subject of the thesis, when this review is appropriate, and (5) a final overall conclusion and/or summary.
- Additional material (procedural and design data, as well as descriptions of equipment used) must be provided where appropriate and in sufficient detail

(e.g. in appendices) to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

- In the case of manuscripts co-authored by the candidate and others, the candidate is required to make an explicit statement in the thesis of who contributed to such work and to what extent; suprvisors must attest to the accuracy of such claims at the Ph.D. Oral Defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to make perfectly clear the responsibilities of the different authors of co-authored papers."

Therefore, I must mention that the contents of sections 3 and 4 are drawn from manuscripts for publication. The manuscript from which section 3 is drawn was co-authored by myself, Chantal Hamel, J.André Fortin, Raymond L. Granger and Donald L. Smith. The manuscript from which section 4 was drawn was co-authored by myself, Chantal Hamel, J.A. Fortin and D.L. Smith.

Donald L. Smith, my supervisor at McGill University, provided funds, assistance and supervisory guidance from the outset of the research to the reviewing of the manuscripts before submission for publication.

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Section 2

GENERAL INTRODUCTION

2.0 Apple production

Since the planting of the first orchard on Mount Royal hillsides, in 1650, the crop has become the most important fruit produced in Quebec. In 1988, the farm value of apple production represented $20 884 000. Following the devastating winter 1980-19981, replanting has favoured the introduction of new varieties, although the cultivar 'McIntosh' remains dominant, representing 75% of the cultivated areas. The replanting has also favoured the production on dwarf and semi-dwarf rootstocks and enhanced the advantages of weed control, with a consequent increase use of herbicides (Maltais, 1985).

2.1 Herbicides

The application of herbicides has became widespread in orchard management, especially with dwarf trees. The suppression of weed competition promotes rapid growth of the young trees and eliminates the difficult task of mowing near trunks with the associated risks of harming trunks and roots. Hence, herbicide utilization has proven to be beneficial both in control of undergrowth and for improved tree performance (Skroch et al., 1975; Heeney et al.,

1981ab; Rupp and Anderson, 1985).

The herbicides currently used in orchard management vary according to many factors such as the year of planting, the target weeds and their developmental stage. For instance, the non-selective herbicide paraquat (Gramoxone), may be used in the year of planting and in subsequent years. Residual herbicides, such as simazine and dichlobenil (Casoron), are recommended for use only after the year of planting. When used alone, they must be applied before the emergence of the weed leaves (Conseil des Productions végétales du Québec, 1987).

2.1.1 Herbicide residues in orchard soils

In general, preplant herbicide incorporation at 1 to 2 kg ha^{-1}, leads to an average accumulation of 1 to 4 ppm, distributed in the surface 4 to 8 cm of soil. Studying the effects of soil-acting herbicides in orchard soils, Clay and Davison (1977) showed that only small quantities penetrate below the top 10 cm of soil. These results are consistent with Skroch's (1975) findings showing that simazine, diuron, terbacil and dichlobenil were present in the top 15 cm but were below the detection level in the 30-60 cm soil layers, a year after application.

2.1.2 Herbicides and VA-mycorrhizae

By their nature, herbicides are designed to antagonize plants and not fungi. It

is not surprising that many studies have reported no effect of various herbicides on fungi, especially when applied at normal dosages. However, after fifty years of increasing use in various crops and ecosystems, herbicides have demonstrated unforseen effects on non target organisms (Trappe et al., 1984). Herbicides have been shown to affect fungi and nematodes as well as plants (Trappe et al., 1984; Edwards, 1989).

The few reports on the effects of herbicides on vesicular-arbuscual (VA) mycorrhizae range from detrimental, neutral to beneficial (Trappe et al., 1984; Edwards, 1989). In a review on herbicide/mycorrhizae interactions, Trappe et al. (1984) have emphasized that some herbicides can drastically affect mycorrhizal fungi and mycorrhizal formation. For instance, Nemec and Tucker (1983) showed that paraquat application, even at much below the registered rate, caused a significant decrease in the percent of citrus root infection. Pope and Holt (1981) measured a detrimental effect of high paraquat dosages on the colonization of ash trees by Glomus fasciculatum (Thaxter) Gerd. and Trappe. They found that the percent root colonization, the production of mycorrhizal hyphae and the viability of the fungus were all reduced.

Certain herbicides have been shown to promote mycorrhizae formation. Nemec and Tucker (1983) observed that simazine increased the percent colonization of citrus roots, when applied at the registered rate. At 5 times the registered

rate, there was a significant decrease in growth and VA mycorrhizal colonization. In Schwab et al.'s (1982) experiment, simazine application even induced VA-mycorrhizae in Chenopodium quinona Willd, an otherwise non mycorrhizal species.

The application rate is clearly an important factor in the effects of herbicides In addition, the formulation, the application technique (foliar versus soil applied), the soil persistence, the soil type, the climatic conditions, the host crop and the VA-fungal species, all affect the response of the mycorrhizal symbiosis to herbicides (Trappe et al., 1984; Edwards, 1989).

Mechanisms proposed to account for effects of pesticides on mycorrhizae include a direct effect on the VA-fungi versus an indirect effect, through the host plant. Since the host plant may often be more sensitive than its associated VA-fungi, particularly when photosynthesis-inhibitory herbicides are used, a plant mediated influence is likely to be more common (Garcia-Romera and Ocampo, 1988). Pesticides may stimulate VA-mycorrhizae through an inhibition of competing soil organisms, or by inducing the host plant to increase the amount of photosynthate allocated to root exudates (Schwab et al., 1982; Nemec and Tucker, 1983).

Variations in herbicide tolerance among VA-fungal species have been

demonstrated (Sieverding and Leihner, 1984). Garcia-Romera and Ocampo (1988) showed that indigenous endophytes were more sensitive to the action of MCPA than G. mosseae (Nicol. and Gerd.) Gerd. and Trappe. At an intermediate application rate, G. mosseae colonization even improved host resistance to MCPA compared to other VA-fungi and non-mycorrhizal controls.

2.2 Vesicular-arbuscular mycorrhizae

It seems that terrestrial plants and mycorrhizal fungi have evolved in association almost from the appearance of vascular plants, since fossil evidence indicates that some of the earliest land plants were mycorrhizal. Fossils of Rhynia and Asteroxylm dated 370 million years old show mycorrhizal associations remarkably like those now found in Psilotum, the closest relative living of these fossil genera (Simon et al. (1993).

The first account of vesicular-arbuscular mycorrhizae in the literature occurs in 1842, when Karl Nageli described hyphae, arbuscules and what seems to be intracellular vesicles, in the roots of iris (Trappe and Berch, 1985). First it was thought that the endophyte had a degenerative influence on the plants, but it was soon noted that the colonization seems to cause little harm to the host.

The term mycorrhiza was first used by Frank in 1885 to describe the symbiotic

association between soil fungi and the roots of a host plant Since then, mycorrhizae have been classified morphologically and anatomically into ectomycorrhizae, vesicular-arbuscular mycorrhizae (VA-mycorrhizae), ericaceous and orchidaceous mycorrhizae (Lewis, 1973).

Vesicular-arbuscular mycorrhizae are by far the most common type and have the widest host range, occuring on almost 90% of vascular plants They are found in liverworts, Pteridophytes, some Gymnosperms and most Angiosperms, but appear to be absent in the mosses (Plenchette, 1982; Harley and Smith, 1983). Gerdemann (1968) listed fourteen families that are never or rarely mycorrhizal, including the Brassicaceae, Chenopodiaceae, Cyperaceae, Juncaceae and Caryophyllaceae. VA-endophytes are believed to be the most abundant fungi in the soil and are so widely distributed that virtually no soils are free from infection. Geographically, they occur from the tropics to the arctic, but the occurrence of a given endophyte can be localized (Mosse, 1978, Plenchette, 1982).

The fungi involved in the VA associations are phycomycetous, with no cross walls and multinucleate hyphae. They are part of the Endogonaceae family, in the class Zygomycetous (Mosse, 1978; Plenchette, 1982). VA species identification is largely based on soil collected spores, with nearly 150 species within six genera currently recognized (Schenck and Perez, 1990)

VA-mycorrhizae are characterized and named after the formation of two intracellular structures formed in the root cortex: vesicles and arbuscules (Mosse, 1978; Maronek et al., 1981; Abbott and Robson, 1982; Plenchette, 1982; Miller et al., 1986). Vesicles are thought to serve as storage organs (Jabaji-Hare et al., 1984), while arbuscules are the site of nutrient exchanges between the fungus and the host-plant (Smith, 1980). These two structures plus the extramatrical hyphae, extending away from the plant root into the surrounding soil, are the visible manifestations of the symbiosis. Unlike some other mycorrhizal fungi, VA-fungi have very little host specificity and can colonize any potential host plant. A single plant may be infected by more than one mycorrhizal fungus, while a single hypha may infect several adjacent plants.

Since early investigations showed apple tree (Malus spp) to be endomycorrhizal (Boulet, 1910), the symbionts involved have been identified as a wide range species of the genera Glomus, Gigaspora, Scutellospora and Acaulospora, in the Endogonaceae family (Miller et al., 1985a). A study of soil samples from a Quebec orchard, revealed the presence of 7 species of Endogonaceae (Dalpé et al., 1986); Glomus constrictum Trappe, Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe and Sclerocystis rubiformis Gerd. and Trappe made up 90% of the mycorrhizal fungi observed. Sampling depth showed that G. constrictum, G. mosseae and S. rubiformis populations were more dense in the

surface layer (0-15cm) than in the deeper layer (15-30cm).

2.2.1 VA-mycorrhiza effects on plants

VA-fungi penetrate host roots, colonize the cortical zone, and trigger changes in plant morphology and physiology (Smith and Gianinazzi-Pearson, 1988). The most documented effect of VA-mycorrhiza is an improved plant growth and nutrition, involving mainly phosphorus. Other major beneficial effects include an improved resistance to diseases caused by soilborne pathogens (Dehne, 1982) and resistance to drought (Runjin, 1989). Overall, VA-mycorrhizae may provide the host plant with an ecologically competitive advantage, facilitating plant survival, growth and nutrition especially under stress conditions (Plenchette et al., 1981).

The plant growth effect has been widely documented on a variety of plants, including citrus (Kleinschmidt and Gerdemann, 1972), tobacco (Daft and Nicholson, 1966), corn (Covey et al., 1981), as well as apple (Plenchette et al., 1981; Koch et al., 1982; Plenchette et al., 1983ab; Hoepfner et al., 1983, Geddeda et al., 1984; Miller et al., 1985a; Gnekow and Marschner, 1989) These effects are generally observed in soils containing low or moderate P levels.

Apples show a strong dependence on VA-mycorrhizae (Covey et al., 1981;

Koch et al., 1982; Geddeda et al., 1984). Hence, experiments conducted in fumigated soils showed that non-inoculated plants were stunted compared to the inoculated treatments (Koch et al., 1982; Geddeda et al., 1984). Both, growth and P uptake were significantly improved in mycorrhizal treatments (Koch et al., 1982). Further studies on P-deficient soils demonstrated that in addition to the increased P content, mycorrhizal apple trees may contain more Cu and Zn, than non mycorrhizal controls (Plenchette et al., 1983a; Gnekow and Marschner, 1989).

Plenchette et al., (1983a,b) observed that mycorrhizal growth enhancement remained obvious at high soil P levels even though the mineral levels of mycorrhizal and non-mycorrhizal apple plants were similar. Such stimulation of plant growth without increased mineral uptake, suggests the possible action of a hormonal mechanism induced in the host by the fungi.

The major mechanism by which mycorrhizal fungi improve P-uptake in mycorrhizal roots is through an extension of the P-depletion zone (Kucey et al., 1989). The physical extensions provided by the extramatrical hyphae increase P-uptake in a soil volume far from the roots, while the hyphae's greater absorbing surface increases uptake close to the roots, as compared to non-mycorrhizal roots (Gray and Gerdemann, 1969; Plenchette, 1982). Owusu-Bennoha and Wild (1980) observed that the depletion zone radius for

mycorrhizal onion roots, was twice that of non mycorrhizal roots.

Though mycorrhizal plants absorb more phosphorus than non mycorrhizal plants, both utilize the same P pool (Sanders and Tinker, 1971; Powell, 1975; Owusu-Bennoha and Wild, 1980; Gianinazzi-Pearson et al , 1981), which is the P available in the soil solution (Tinker, 1975; Gianinazzi-Pearson et al , 1981).

VA-mycorrhizal plants show no capacity to directly solubilize insoluble phosphate such as rock phosphate, but they may aid plants utilizing such sources (Mosse et al., 1976; Mosse, 1977). This ability is a consequence of their increased efficiency to exploit the soil solution (Gerdemann, 1975), which leads to an increased mobilization of P from the solid phase into solution (Kucey et al., 1989).

2.2.2 VA-mycorrhizae efficiency

Though most VA-fungi are capable of forming a symbiosis, to at least some degree, with most potentially mycorrhizal plants (Mosse, 1978; Harley and Smith, 1983), there are differences in the efficacy of the symbioses (Abbott and Robson, 1978; Abott and Robson, 1981). Experimental evidence shows that considerable variability exists between species and even cultivars to different VA-endophytes (Miller et al., 1986). For instance, Granger et al.,

(1983) found that inoculation of Glomus versiforme (Karsten) Berch significantly increased growth of Malling 7 apple rootstock while Malling Merton 11 growth was not increased. In addition, the edaphic conditions play a major influence in the establishment of the symbiosis. Hence, certain host, fungi and soil combinations seems more efficient in promoting apple growth (Benson and Covey, 1976, Covey et al., 1981; Plenchette et al., 1981; Hoepfner et al., 1983; Miller et al., 1985a; Miller et al., 1989).

2.3 Hypotheses and Objectives

The first hypothesis of this research was that apple rootstock cultivars respond differently to VA-fungi species or isolates and that some cultivar/VA-fungi combinations are more efficient in promoting apple growth than others.

The specific objectives related to this hypothesis were :

. to compare growth, nutrition, dry mass production and leaf surface area of rootstock cultivars inoculated with VA-fungi and identify the best combination.

. use the Diagnosis Recommendation Integrated System (DRIS) to interpret plant nutrient analysis data.

. measure the percent root colonization and the extramatrical hyphal network and determine their relationship to the efficiency of the symbiosis.

The second hypothesis was that soil applied herbicides affect directly VA-spore germination and that mycorrhizal apples respond differently to herbicides than non-mycorrhizal apples.

The specific objectives related to this hypothesis were:

. to axenically grow VA-spores in vitro on transformed roots and test herbicide treatments on spore germination.

. to study the effects of application of herbicides after establishment of the symbiosis on apple.

. to compare plant responses to herbicide application in terms of growth, photosynthetic rate and mortality, in mycorrhizal versus non-mycorrhizal apple plants.

. to measure and compare the percent root colonization in herbicide treated versus control plants.

Preface to Section 3

Section 3 was taken from the manuscript by Morin, Hamel, Fortin, Granger and Smith submitted to the Journal of American Society for Horticultural Science. The format has been changed to conform to a format consistent within this thesis. The literature cited in this section is listed in the reference section. Each table or figure is presented at the end of this section.

In this section, we evaluated the efficiency of several VA-fungi/apple rootstock combinations. Advantages of early inoculation of apple rootstocks are presented and identification of the best fungus set the stage for the second part of the research, testing the effects of herbicides on the symbiosis with this fungus.

Section 3

Apple rootstock response to vesicular-arbuscular mycorrhizal fungi in a high phosphorous soil.

3.0 Abstract

The efficiency of the mycorrhizal symbiosis may vary with combinations of cultivars and mycorrhizal isolates. A 12-week greenhouse experiment was undertaken to test the efficiency of four isolates from three species of vesicular-arbuscular mycorrhizal fungi on four apple (Malus domestica Borkh) rootstock cultivars: M.26, Ottawa 3 (Ott.3), P.16 and P.22. The plants were grown in a soil from an apple rootstock nursery, containing a high level of available P (644 kg-Bray 1 ha^{1}). The inoculation treatments were: Glomus aggregatum Shenck and Smith emend. Koske, G. intraradix Shenck and Smith and two isolates of G. versiforme (Karsten) Berch, one originally from California (CAL) and the other one from Oregon (OR). Mycorrhizal plants were taller, produced more biomass and had a higher leaf P concentration than the control plants. Mycorrhizal inoculation also significantly increased the leaf surface area of M.26 and Ott.3 compared to the controls. G. versiforme (CAL)-inoculated plants tended to have the best nutrient balance, the greatest final height and shoot biomass, and produced an extensive hyphal network. All the mycorrhizal plants had similar percentages of root colonization but the size of the external

hyphal network varied markedly with the fungal species. G. versiforme (OR) had a larger extramatrical phase than G. aggregatum and G. intraradix. Mycorrhizal efficiency was associated with a larger external hyphal network but showed no relation with the internal colonization. Interactions between fungal species and rootstock cultivars indicated that while M.26 responded better to G. versiforme (CAL) in terms of plant height at 4 and 12 weeks and to leaf area production, Ott.3 tended to produce more leaf area with G. versiforme (OR). Despite the high P fertility of the soil used, growth enhancement due to mycorrhizal inoculation was attributed to an imporved P nutrition.

3.1 Introduction

Apple trees show a strong dependency on mycorrhizae (Covey et al , 1981; Koch et al., 1982) and in orchards they form symbioses with the naturally occurring vesicular-arbuscular mycorrhizal (VAM) flora (Miller et al., 1985a; Dalpé et al., 1986). Mycorrhizae benefit apple plants through an improved growth and nutrition (Plenchette et al., 1983ab; Hoepfner et al , 1983; Geddeda et al., 1984; Miller et al., 1985b) involving mainly P and in some cases, other immobile nutrients such as Zn and Cu (Gnekow and Marschner, 1989). Other beneficial effects include an improved resistance to drought (Runjin, 1989) and diseases caused by soilborne pathogens (Dehne, 1982).

Under unsterilized P-poor field conditions, Plenchette et al (1981) showed that inoculation of greenhouse-produced apple seedlings, prior to field planting, can significantly increase growth as compared to both phosphorus-fertilized and unfertilized naturally mycorrhizal controls. These results indicate the benefit in planting apple plants pre-colonized with a highly compatible VAM-fungus, even if the plants are to become colonized by the indigenous VAM-flora, once in the field.

Host plants show inter- and intraspecific variations in their ability to form and benefit from association with a VAM fungus. Experiments on wheat (Azcon and Ocampo, 1981; Young et al., 1985; Manske, 1990), soybean (Heckman and

Angle, 1987), maize (Toth et al., 1984), millet (Krishna et al., 1985); and groundnut (Kesava et al., 1990) showed differences in percent VAM colonization and growth response between parents and progenies tested, suggesting the posssibility of breeding plants for improved mycorrhizal associations (Gianinazzi and Gianinazzi-Pearson, 1990).

The fungal species or isolate also influences the efficiency of the symbiosis in apple (Geddeda et al., 1984; Miller et al., 1985b). For instance, Benson and Covey (1976) reported that inoculation of apple seedlings with Glomus fasciculatum (Thaxter) Gerd. and Trappe emend. Walker and Koske in a fumigated soil caused a greater growth stimulation than that of G. mosseae (Nicholson and Gerdemann) Gerdemann and Trappe. Similarly, Miller et al. (1985b) showed that in a low P-soil, seven isolates had different effects on apple growth.

Mycorrhizal efficiency depends not only on the host plant and the VAM-fungus, but also on edaphic conditions, particularly on soil P fertility. For instance, Hoepfner et al (1983) found that infection levels of G. mosseae (Nicholson and Gerdemann) Gerdemann and Trappe on apple seedlings ranged from 11 to 41 %, depending on the soil tested. Hence, any experiment testing the practical use of inoculation must take the soil component into consideration as well as the mycorrhizal partners. As apple orchard soils are fertile or fertilized,

evaluation of mycorrhizal effects on apple should not be conducted under low P availability.

Specific combinations of fungal isolates and apple cultivars could be better than others. As the benefit of the symbiosis to the plant comes from the more efficient exploration of the soil volume by mycorrhizal hyphae as compared to roots (Gnekow and Marschner, 1989), the best isolate-cultivar combinations should be characterized by the production of an abundant extramatrical mycelium and a high shoot:root ratio. This experiment was undertaken to test the efficiency of four isolates of three VAM-fungi on four apple rootstock cultivars grown in a P rich soil, and to determine the relationship between efficiency and extent of mycorrhizal colonization with particular reference to external hyphal network.

3.2 Materials and Methods

A factorial experiment (five inoculation treatments x four rootstock cultivars) was conducted under greenhouse conditions for 12 weeks. Four apple (Malus domestica Borkh) rootstock cultivars (Malling 26 [M.26], Ottawa 3 [Ott.3], P.16 and P.22) were inoculated with each of four VAM-isolates, or were not inoculated. The experiment was a split-plot randomized in five complete blocks. The rootstock cultivars were randomized to the main plots while the

inoculation treatments were randomized to the subplots.

A total of 100 micropropagated, non-mycorrhizal apple rootstocks were planted individually in 6-inch plastic pots, in a sandy loam soil taken from the top 20 cm of the soil profile in an apple rootstock nursery, at St-Jean-Baptiste-de Rouville, Québec. The soil contained 2.2 % organic matter and the fertility status at the beginning of the experiment was: pH = 7.3, 644 kg of P-Bray 1 ha^{-1}, 2380 kg of K ha^{-1}, 3090 kg of Ca ha^{-1}, 697 kg of Mg ha^{-1}, 1.6 ppm B, 2.2 ppm Cu, 360.1 ppm Fe, 34.8 ppm Mn, 12.8 ppm Zn. The soil was passed through a 5-mm sieve and steam pasteurized at 72°C for 5 hours. This treatment allowed for destruction of the indigenous mycorrhizal fungi, while retaining a portion of the microflora. To alleviate the loss of texture due to manipulation, the soil was amended with grade 16 silica sand (1 part of sand: 3 part of soil, v/v).

Inoculation.

The fungi tested were: two isolates of Glomus versiforme (Karsten) Berch, one from California (CAL), the other from Oregon (OR) both isolated from unknown hosts plants, G. aggregatum Shenck and Smith emende Koske isolated from an alfalfa field in Lapocatière, Quebec, Canada, and G. intraradix Shenck and Smith, isolated from an ornamental plant production field in Pont Rouge, Quebec, Canada. The inocula consisted of leek (Allium porrum L.) roots

colonized with one of each of the above fungi. One gram (fresh mass) of inoculum was placed at the bottom of the transplant hole, immediately prior to planting. Control plants received one gram of an autoclaved mixture of all of the above inocula. To account for effects of nonmycorrhizal organisms potentially found in the inocula, all plants also received 25 ml of filtered (Whatman GF/D, 2.7 um) washings of a mixture of the fungal inocula.

Plants were given small amounts of water, to avoid dripping No fertilization was performed.

Parameters studied

Every two weeks, plant heights were measured to the closest 0.5 cm At the end of the experiment, the plants were harvested, and the leaf area of each plant was measured with a Delta-T Area Meter System (Delta-T Devices Ltd, Cambridge, England). Roots, leaves and stems were dried at 70°C for 48 hours and weighed.

Dried leaves were ground and ashed for mineral analysis. To determine N and P contents, tissue samples were digested according to the method of Isaac and Johnson (1976) and the nutrient concentrations were determined colorimetrically (methods 825-87T and 862-87T, Bran and Lubbe Cie) The concentrations of the other minerals were determined after nitric acid digestion

of the tissues (Havlin and Soltanopour, 1980) and measured using an inductively-coupled argon plasma spectrometer. DRIS (Diagnosis and Recommendation Integrated System) indices were calculated from the leaf mineral contents (Parent and Granger, 1989).

A fresh root sample of each plant was cleared with 2.5 % KOH and stained with acid fuschin (Brundrett, Piché and Peterson, 1984) to estimate their mycorrhizal colonization status. The percentage of root length containing vesicles was assessed by the grid intersect method (Giovanetti and Mosse, 1980).

After harvesting the roots, the soil from each pot was collected separately and mixed thoroughly to homogenize the extramatrical hyphae. A 5-g sample of soil containing hyphae was suspended in 200 ml of water and a 15 ml subsample of the soil suspension was collected on a filter (Whatman no 1). The external hyphae were stained with a solution of acid fuschin (2 % in 85 % lactic acid) and rinsed with acidified water. The hyphal length was measured by the modified line intersect method of Tennant (1975), based on three subsamples per pot.

Statistical analysis

An analysis of variance (ANOVA) was run on the data and the differences

between the treatment means were further determined with the Bonferroni multiple range test. Data for the leaf mineral concentrations were analyzed after arcsin transformation, except for B, Zn, and Mn which did not require transformation.

3.3 Results

Plant height

After six weeks, plants inoculated with G. aggregatum, G. versiforme (OR) and G. intraradix were taller than the controls (Fig. 3 1). Plants inoculated with G. versiforme (CAL) were significantly taller than the controls only by the 8th week. From the 8th to the 10th week, the mycorrhizal plants were twice as tall as the control plants; for example, at the 10th week the average heights were 17.9 cm compared to 8.7 cm respectively, for inoculated plants and controls. By the end of the experiment, the growth of the mycorrhizal plants had reached a plateau, with the result that plants inoculated with G. intraradix and G. aggregatum were no longer significantly taller ($P>0.05$) than the control plants. G. versiforme (CAL)-inoculated plants tended to have the greatest final height. At the 4th and 12th week, a significant interaction ($P<0.05$) between cultivar and inoculation indicated a better response of M.26 rootstock to inoculation, as compared to the other cultivars.

Leaf surface area

A significant ($P < 0.01$) cultivar x inoculation interaction revealed that the leaf area of Ott.3 and M.26 rootstocks increased in response to inoculation while that of P.16 and P.22 did not (Fig. 3.2). In addition, M.26 tended to produce the greatest leaf surface area when inoculated with G. versiforme (CAL).

Dry mass

The fungal isolates had similar effects on plant root and shoot dry mass (Fig. 3.3), as in general, mycorrhizal plants produced significantly greater dry weights than the control plants. Plants inoculated with G. versiforme (CAL) had a greater ($P < 0.05$) shoot/root dry mass ratio than that of G. intraradix and G. aggregatum (data not shown). There was no difference between the shoot/root ratios of plants inoculated with the two G. versiforme isolates.

Mineral analysis

The N, Mg, S and Fe leaf contents of mycorrhizal plants were similar among the mycorrhizal treatments, and significantly lower than that of the control plants (Table 3.1). In contrast, the P level of control plants was significantly lower than that of the mycorrhizal plants and was below the critical value of 1.3 mg g^{-1} determined by Shear and Faust (1980). N was deficient in plants of all mycorrhizal treatments as the concentrations were below the critical value of 15 mg g^{-1} (Shear and Faust, 1980). The negative DRIS indices (Table 1)

emphasize that N was limiting (Kelling and Schulte, 1986). Although plant K and Ca concentrations were above their respective critical level, the DRIS indices showed that they tended to be limiting in the control treatment. Mg was in the sufficiency range for optimum yield by either approach, for all the inoculation treatments. Overall, the mycorrhizal plants had a better nutrient balance as shown by the lower sum of DRIS-index values (Jones et al., 1986; Granger and Parent, 1988) as compared to the control plants. The plants colonized by G. versiforme (CAL) showed the best nutrient balance of all inoculation treatments.

Mycorrhizal colonization

At the end of the experiment, P.16, M.26 and Ott.3 were highly mycorrhizal, with over 90 % of their root length colonized, while P.22 was significantly ($P<0.05$) less colonized than M.26 and Ott.3, with 81% root colonization (Fig. 3.4).

Plants of mycorrhizal treatments had similar percentages of root colonization, which varied from 88 to 96 % (Fig. 3.5). No mycorrhizal colonization was observed in the non-inoculated control plants.

Extramatrical hyphal length

Cultivars had similar ($P>0.05$) lengths of external hyphae (Fig. 3.4), but G.

versiforme produced more external mycelium than G. intraradix and G. aggregatum and there was no significant difference between the two isolates (Fig. 5). The presence of some extramatrical hyphae in the soil of the controls, can be explained by the fact that the staining and measuring methods did not discriminate between VAM fungi and other fungal mycelium. However, the amount of extramatrical hyphae in the controls was small compared to that of the inoculated plants (<10 %).

3.4 Discussion

Despite the high P content of our soil, all mycorrhizal isolates improved P nutrition and apple growth, compared to the non-inoculated controls. This positive effect on plant growth is in agreement with the studies of Gnekow and Marschner (1989) and Plenchette et al. (1983ab), in which mycorrhizal growth enhancement of apples remained significant in substrates containing high levels of available P. In contrast with the latter studies, our mycorrhizal plants contained a higher leaf P concentrations than the non-mycorrhizal plants, while Zn and Cu concentrations remained statistically similar to the control plants. Such an increase in leaf P-content is commonly observed in mycorrhizal plants growing in P-deficient soils (Koch et al., 1982; Plenchette et al., 1981), but is not expected under conditions of high P fertility. Nevertheless, in spite of the high P availability of our soil (644 kg ha^{-1}), the improved growth of the

mycorrhizal plants was probably due to a better P nutrition, as indicated by the leaf mineral analysis. These results emphasize that the P-nutrition of apple trees is very dependant on the mycorrhizal symbiosis. Therefore, responsiveness to mycorrhizal colonization is a characteristic which should be considered in the evaluation of apple rootstocks.

G. versiforme (CAL) was the best fungal isolate. It produced an extensive extramatrical phase and the plants inoculated with this isolate had the best nutrient balance (lowest total DRIS index), greatest final height, shoot dry mass and shoot/root ratio. The large shoot/root ratio of G versiforme (CAL) inoculated plants can be related to their extensive extramatrical hyphal network, which lowered the need for root production. Sanders et al., (1977) also reported a decrease in the onion shoot/root ratio with effective endophytes which they related to improved P nutrition.

It is unlikely that the initial delay of G. versiforme (CAL) in promoting plant growth, compared to the other mycorrhizal fungi, was a result of a delayed onset of colonization as studies on the colonization process have shown that the most efficient VAM-species produced the highest and most rapid rate of colonization (Abbott and Robson, 1981; Reich, 1988; Miller, Bodmer et al., 1989). The initial delay in plant response may have been due to a greater drain on photosynthates by G. versiforme (CAL), during the development of a

particularly extensive external mycelium, which finally was found to be very efficient. This hypothesis, however, needs to be verified.

A number of investigators (e.g. Krishna et al., 1985; Kesava et al., 1990) have demonstrated that the reponsiveness of host plant to mycorrhizal inoculation could be increased through plant breeding. Although G. versiforme (CAL) and M.26 formed the best plant-fungus combination in terms of leaf surface area and in spite that in general, M.26 plants responded better to the mycorrhizal treatments than the other cultivars, the differences were slight and not statistically significant.

The depressed growth rate of mycorrhizal plants observed near the end of the experiment was due to a N deficiency, as a result of a dilution effect, i.e. plant growth surpassed N uptake. Mycorrhizal plants grew bigger and depleted soil N, which became limiting. Hence, it can be expected that in the presence of a sufficient N supply, the exponential growth of the mycorrhizal plants would have lasted longer and the final gap between them and the controls would have been more pronounced. In contrast, the P-deficient control plants did not grow enough to reach this level of soil N depletion. The data clearly show that, in spite of the high P availability of the soil, non-mycorrhizal plants were P limited.

At the end of the experiment, the fungal isolates differed in the length of their

extramatrical hyphae, while they did not show any significant difference in their percentage of internal root colonization. Abbott and Robson (1985), working on clover, also found that VAM-species differed in the length of extramatrical hyphae produced per cm of colonized root.

Although P.22 had a significantly lower percent colonization than M.26 and Ott.3, all the rootstocks showed similar growth responses to mycorrhizal inoculation. These results indicate that growth enhancement is more related to the extent of the extramatrical phase than to internal root colonization. Mycorrhizal efficiency seems characterized by an extensive external hyphal network and it appears that fungal isolates should be selected on the basis of this character, probably along with others such as their ability to colonize plants rapidly (Abbott and Robson, 1981).

In conclusion, in our P-rich soil (644 kg ha^{-1}), the benefit to mycorrhizal inoculation was attributed to a better P nutrition. Although M.26 rootstock tended to respond better to mycorrhizal inoculation, the interaction was rather weak. G. versiforme, especially the Californian isolate, was best for growth promotion and nutrition of all apple rootstocks. Large extramatrical mycelium development was associated with mycorrhizal efficiency.

Table 3.1 Leaf mineral concentrations and DRIS[z] indices of apple rootstock cultivars inoculated with different VAM isolates

Inoculation treatments	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	mg g-						ug g-1				
G. versiforme(OR)	13.0[y]a	1.90 a	23.1 a	10.6 a	3.37 a	1.67 a	55.4 a	16.0 a	142.9 a	48.6 a	41.4 a
G aggregatum	12.7 a	2.30 a	21.6 a	10.8 a	3.18 a	1.54 a	57.1 a	18.0 a	142.4 a	48.0 a	43.6 a
G. intraradix	12.4 a	2.01 a	21.8 a	10.6 a	3.23 a	1.65 a	56.6 a	15.7 a	137.8 a	50.2 a	46.2 a
G. versiforme(CAL)	14.3 a	1.96 a	22.7 a	10.2 a	3.37 a	1.72 a	55.8 a	17.2 a	138.8 a	47.2 a	40.6 a
Control	18 9 b	1.24 b	23.1 a	10 8 a	3.84 b	2.04 b	60 5 a	15.2 a	180.4 b	50.3 a	43.7 a

	DRIS index[x]					Total DRIS index
G. versiforme(OR)	-36	9	0	-14	41	100
G. aggregatum	-36	9	5	-12	35	97
G. intraradix	-36	9	3	-14	38	100
G. versiforme(CAL)	-33	7	0	-13	38	91
Control	-15	-5	-18	-18	56	112

[z] DRIS = Diagnosis and Recommendation Integrated System.

[y] Values are means of 20 replicates. Within a column, means followed by the same letter are not significantly different ($P<0.05$, Bonferroni test).

[x] The most negative index is for the element most required and the most positive index gives the element least required (Kelling and Schulte, 1986).

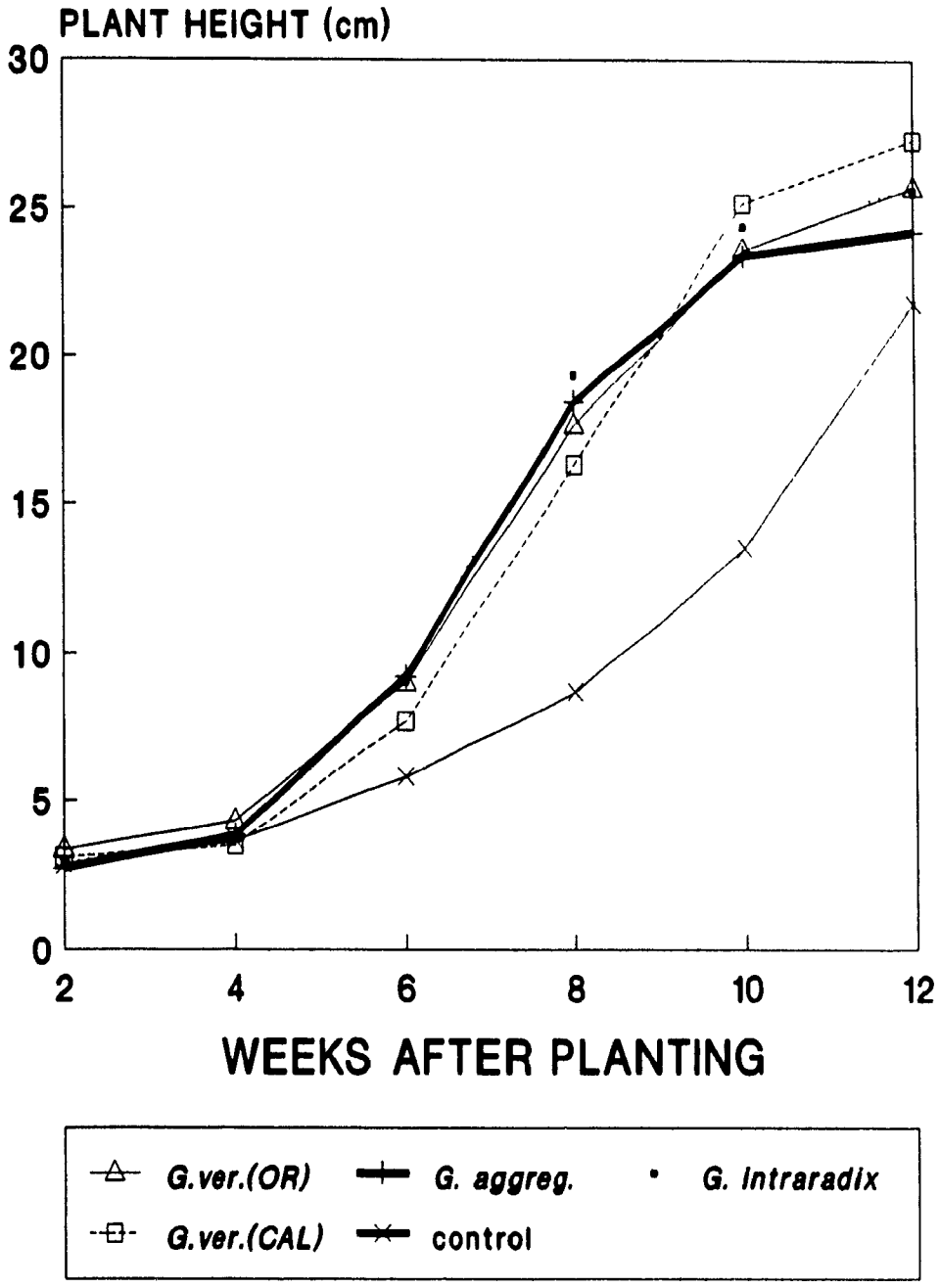

Figure 3.1 Height of four apple rootstock cultivars grown in a nursery soil under greenhouse conditions over twelve weeks. Values are means of 20 replicates. The bar represents the range at $P<0.05$ (Bonferroni test)

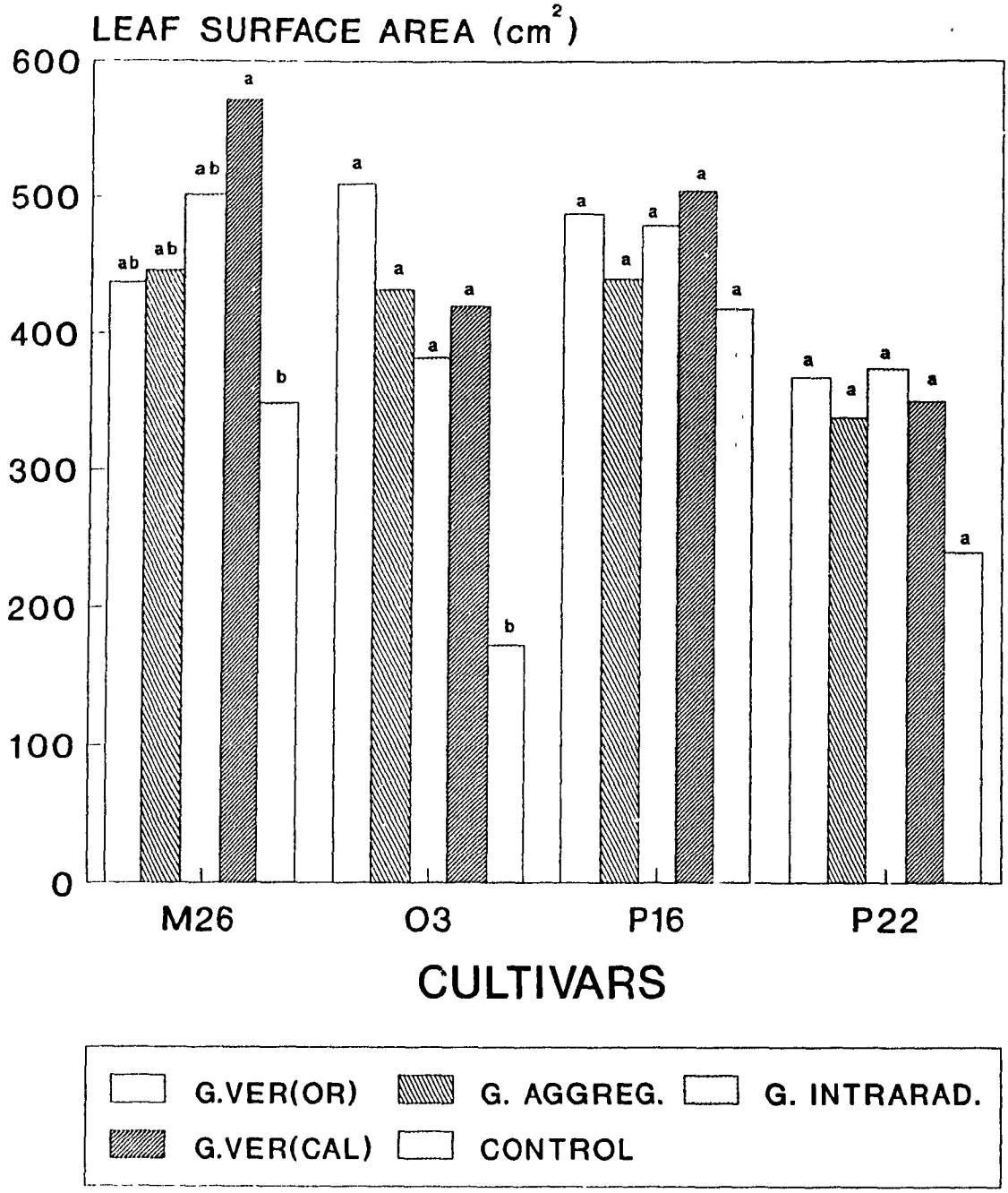

Figure 3.2 Leaf surface area of four apple rootstock cultivars, grown in a nursery soil under greenhouse conditions. Values are means of 5 replicates. Within rootstocks, bars with the same letter are not significantly different ($P<0.05$, Bonferroni test).

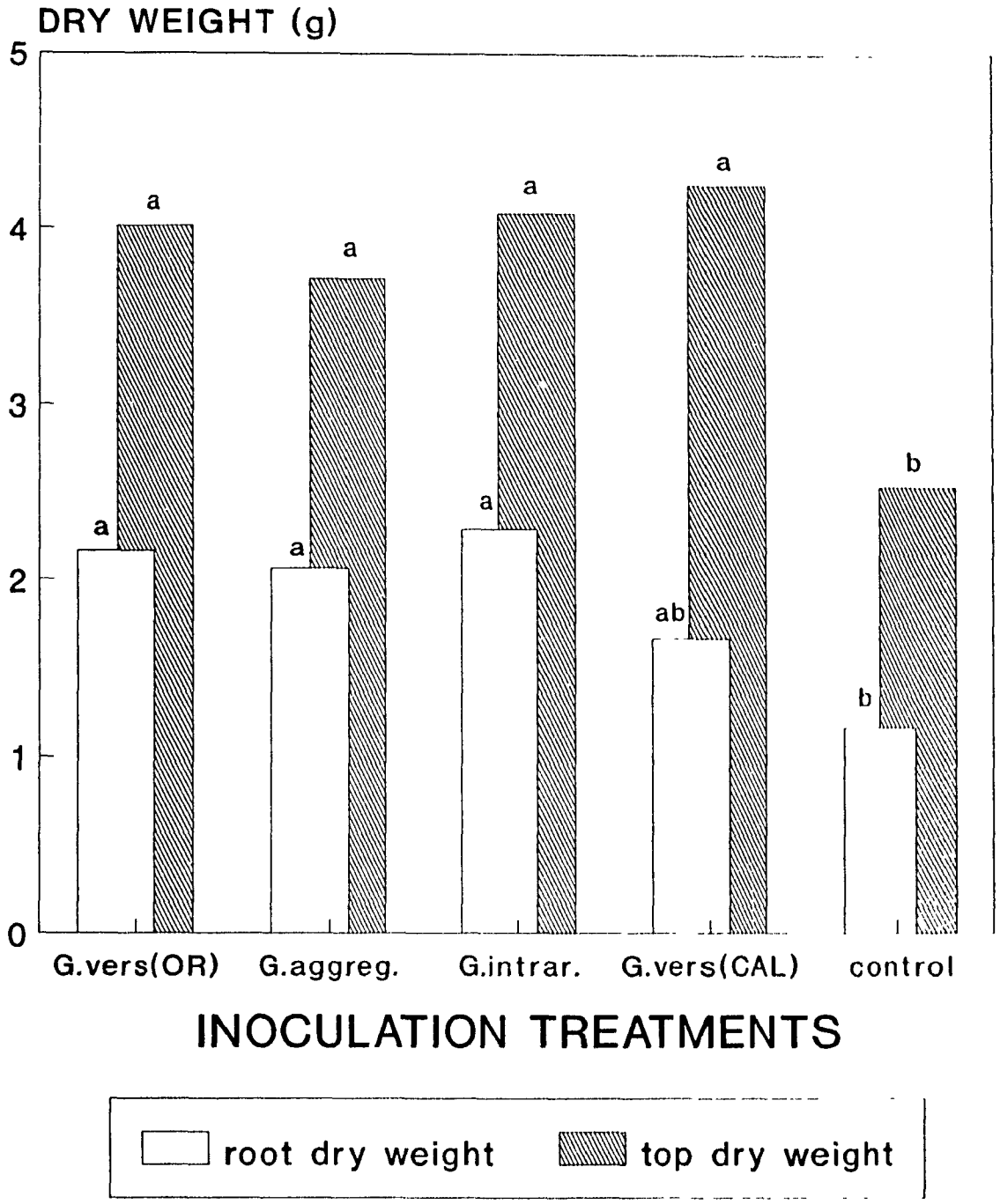

Figure 3.3 Shoot and root dry weights of apple rootstock cultivars, inoculated with different VAM isolates and grown in a nursery soil under greenhouse conditions. Values are means of 20 replicates. Within a series, bars with the same letter are not significantly different ($P<0.05$, Bonferroni test).

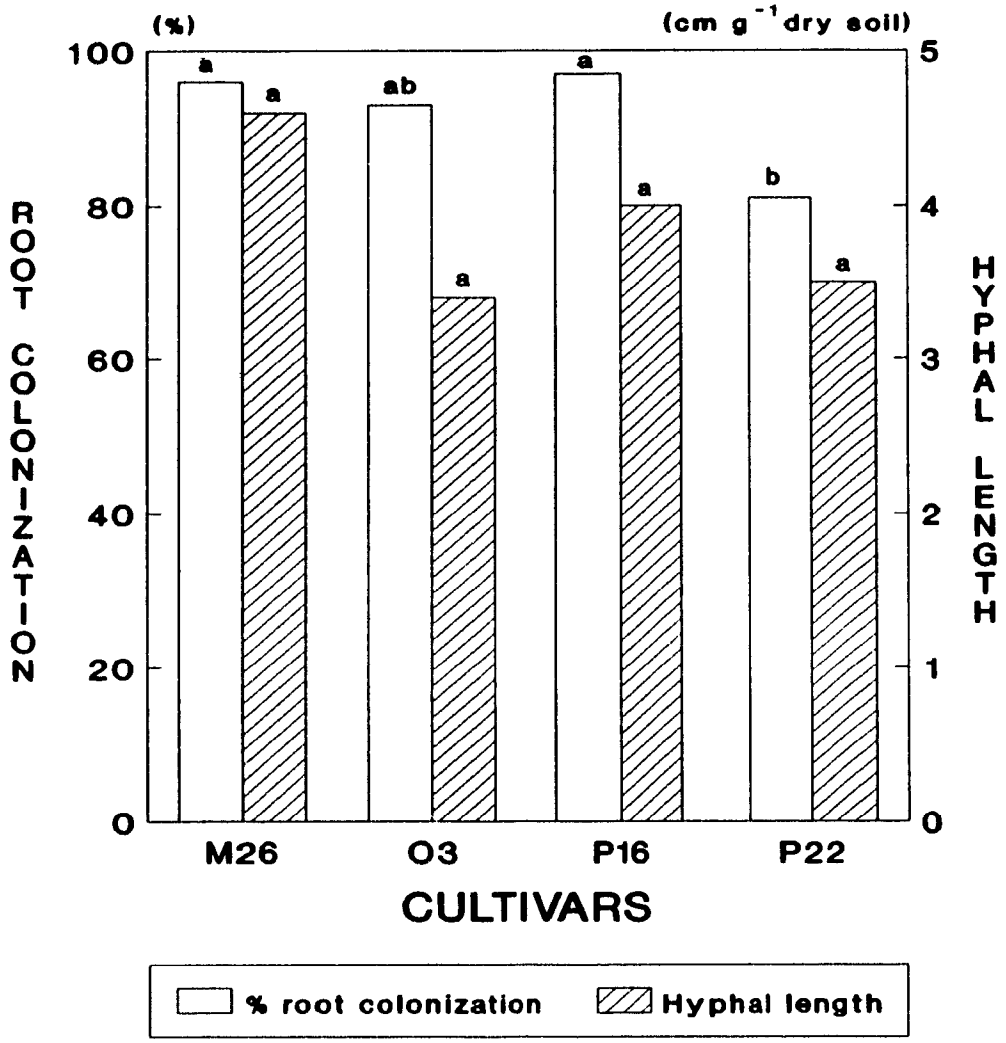

Figure 3.4 Percentage root colonization and extramatrical hyphal length of four apple rootstock cultivars grown in a nursery soil under greenhouse conditions. Statistical analysis for root colonization does not consider non-mycorrhizal controls, since all values were zeros. Bars are means of 25 replicates. Within a series, bars with the same letter are not significantly different ($P < 0.05$, Bonferroni test).

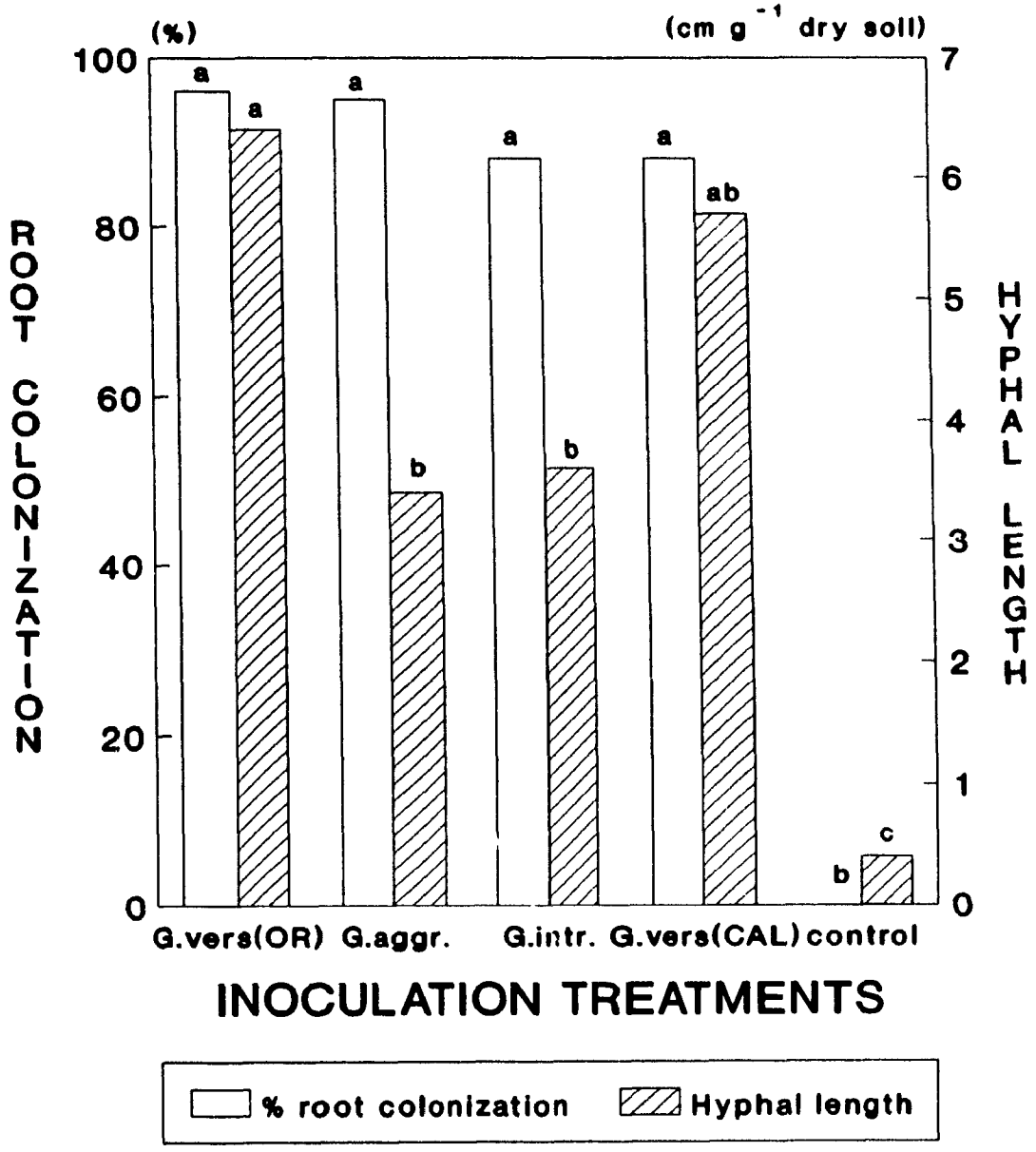

Figure 3.5 Percentage root colonization and extramatrical hyphal length of four apple rootstocks inoculated with different VAM isolates. Values are means of 20 replicates. Within a series, bars with the same letter are not significantly different ($P < 0.05$, Bonferroni test).

Preface to Section 4

Section 4 was taken from the manuscript by Morin, Hamel, Fortin and Smith submitted to the Journal of American Society for Horticultural Science. The format has been changed to conform with the one used in this thesis. The literature cited in this section is listed in the reference section. Each table or figure is presented at the end of this section.

In section 3, dependency of apple rootstocks on various VA-fungi was characterized and G. versiforme (CAL) was identified as the best performing fungus. In section 4, we examine the effects of herbicides currently used in orchards on established apple mycorrhizae, with G. versiforme (CAL). We also evaluate herbicide effects on spore germination in vitro.

Section 4

Mycorrhizal colonization increases herbicide toxicity in apple

4.0 Abstract

Herbicides are increasingly used in orchards. Since apple trees are strongly dependent on mycorrhizae, we have examined the effect of three commonly used herbicides on the host plant and on the endophyte. The symbiosis was allowed to establish for 8 weeks on tissue-cultured P16 apple rootstocks inoculated with Glomus versiforme (Karsten) Berch under greenhouse conditions. Simazine (1, 2, 10 and 20 ug a.i. g^{-1}), dichlobenil (1, 5, 10 and 25 ug a.i. g^{-1}), paraquat (0.5, 1, 10 and 100 ug a.i. g^{-1}) or water was then applied to mycorrhizal and non-mycorrhizal plants as soil drench. The response of mycorrhizal plants to herbicidal applications was larger, and the relative elongation rate was more sharply reduced in mycorrhizal plants (76%) than in non-mycorrhizal plants (33%). Six weeks after application, dry mass reduction due to herbicides were similar (39% and 36%) for mycorrhizal and non-mycorrhizal plant shoots, respectively, while reduction in root dry mass was larger for mycorrhizal plants (63%) compared to non-mycorrhizal plants(46%). A spore germination test run with G. intraradix on herbicide amended (0, 1, 10, 100 and 1000 ug a.i. g^{-1}) water-agar had shown that either dichlobenil or paraquat, even at the lowest concentration, could reduce germination in vitro; none of the herbicide treatments affected root colonization. Simazine did not

affect hyphal elongation in vitro suggesting that the well known improved absorption capacity of mycorrhizae could explain, at least in part, the increased phytotoxicity of the herbicides, since it was found that plant mortality was higher among mycorrhizal than non-mycorrhizal apple trees for all the herbicidal treatments. The increased CO_2 assimilation rates of dichlobenil treated mycorrhizal plants, contrasting with the decreased CO_2 assimilation rates of control plants measured 1 week after dichlobenil treatment, indicates a physiological interaction between mycorrhizal colonization and dichlobenil in the toxic response of apple plants.

4.1 Introduction

An intimate relationship between vascular plants and arbuscular mycorrhizal fungi has developped during the course of 400 million years of coevolution (Simon et al., 1993). The arbuscular endophytes are so widely distributed that virtually no soils are free from them (Mosse, 1978), and most plants form arbuscular mycorrhizae (Harley and Smith, 1983). Apple (Malus domestica Borkh) is no exception. Since the early investigations showing apple to be mycorrhizal (Boulet, 1910), a wide range of mycorrhizal fungal species were found in symbiosis with apple trees (Dalpé et al., 1986; Miller et al., 1985).

Mycorrhizal fungi are normal component of apple plant root systems. The arbuscular mycorrhizal symbiosis of apple insures a good uptake of nutrients, particularly phosphorus, zinc, and copper (Benson and Covey, 1976; Covey et al., 1981; Gnekow and Marschner, 1989), and the mycorrhizal symbiosis improved apple plant resistance to drought (Runjin, 1989) and to diseases caused by soilborn pathogens (Dehne, 1982). Apple shows a strong dependance on mycorrhizae (Covey et al., 1981, Geddeda et al., 1984), and growth enhancement of the plant due to mycorrhizae remains obvious even at high soil P-levels (Plenchette et al., 1983ab)

The application of herbicides has become a wide spread practice in orchard management, especially with dwarf trees, and it now seems that some

herbicides can drastically affect arbuscular fungi and mycorrhizae (Trappe et al., 1984). Some herbicides can affect arbuscular fungi either directly or through their effects on the host plants (Garcia Romera and Ocampo, 1988). One such herbicide, simazine, induced mycorrhizal fungal penetration in Chenopodium quinona, an otherwise non-mycorrhizal species (Schwab et al., 1982). Most of the time, however, herbicides have either no effect (Burpee and Cole, 1978; Ocampo and Barea, 1982; Smith et al., 1981; South, 1981) or affect negatively the mycorrhizal symbiosis (Nemec and Tucker, 1983; Pope and Holt, 1981; Sieverding and Leihner, 1984).

Paraquat, a non-selective herbicide, is recommended for application in orchard the year of planting and in subsequent years (Conseil des Productions Végétales du Québec, 1987). The residual herbicides simazine and dichlobenil are recommended for use after the planting year. Therefore, we have tested the effect of increasing rates of paraquat, simazine and dichlobenil on mycorrhizal apple rootstocks and on elongation, in vitro, of mycelium from spore clusters of a Glomus sp.

4.2 Materials and Methods

Pot experiment

A 2x13 factorial experiment was run under greenhouse conditions. Tissue-cultured P16 apple rootstocks either colonized by the arbuscular

endomycorrhizal fungus Glomus versiforme, or kept uninfected were treated with four levels of simazine (2-chloro-4,6-bis-ethylamino-s-triazine; 1, 2, 10 and 20 ug a.i. g^{-1}), dichlobenil (2,6-dichlorobenzonitrile; 1, 5, 10 and 25 ug a.i. g^{-1}), paraquat (1,1'-dimethyl-4,4'bipyridinium; 0.5, 1, 10 and 100 ug a.i. g^{-1}) or with water. Herbicide concentrations had been selected to include the recommended rates (2 ug a.i. simazine, 5 ug a.i. dichlobenil and 0.5 ug a.i paraquat g^{-1} soil) according to the Conseil des Productions Végétales du Québec (1987), and levels of toxicity already reported in the literature (Dvorak, 1968, Hogue and Neilsen, 1988; Lord et al., 1972; Lord and Greene, 1975, Robinson and Lord, 1970). The herbicides treatments were applied as soil drench, and were replicated in four blocs.

Apple rootstocks were planted in 4 L pots containing a sandy loam soil taken from the top 20 cm of the soil profile in an apple rootstock nursery, at St-Jean-Baptiste-de-Rouville, Québec. The soil contained 2.2 % organic matter and the fertility status at the beginning of the experiment was: pH = 7.3, 644 kg of P-Bray 1 ha^{-1}, 2380 kg of K ha^{-1}, 3090 kg of Ca ha^{-1}, 697 kg of Mg ha^{-1}, 1 6 ppm B, 2.2 ppm Cu, 360.1 ppm Fe, 34.8 ppm Mn, 12.8 ppm Zn. The soil was passed through a 5-mm sieve and steam pasteurized at 72°C for 5 hours. This treatment allowed for destruction of the indigenous mycorrhizal fungi. To alleviate the problem of structure loss due to manipulation, the soil was amended with grade 16 silica sand (1 part sand: 3 parts soil, v/v).

Half of the plants were inoculated with 1 g (fresh mass) of corn roots colonized by Glomus versiforme and cut into 1 cm fragments. The inoculum was placed at the bottom of the transplant holes, immediately prior to planting. Control plants received one gram of autoclaved inoculum. To account for the effects of non-mycorrhizal organisms possibly carried in the inoculum, control plants also received 5 ml of filtered (Whatman GF/D, 2.7 um) washings of the mycorrhizal inoculum.

The symbiosis was allowed to establish for 8 weeks before application of the herbicide treatments. The different herbicide treatments were prepared in 500 ml of distilled water and applied as soil drench to the designated pots.

Plants were always watered according to their needs, giving just enough water to avoid dripping. They were fertilized once, after 6 weeks, with a phosphate-free Long Ashton nutrient solution to alleviate an anticipated nitrogen deficiency.

Measurement of plant photosynthetic rates was made to evaluate the impact of the herbicide treatments 1 week after their application, using a Licor-6200 photosynthesis meter (Li-Cor, Lincoln, NB, USA). The photosynthetic activity of the penultimate fully expanded leaf of each plant was recorded in duplicate.

Plant height was measured every other week. Six weeks after herbicide treatment, mortality was recorded and plants were harvested. Plant dry mass was recorded after drying at 70°C for two days and percentage of root colonization was measured using the grid intersect method (Giovanetti and Mosse, 1980) after staining root samples with acid fuschin (Brundrett et al., 1984).

Spore germination test

Spore clusters (for a total of 20-30 spores) of in vitro-cultured (Bécard and Fortin, 1988) Glomus intraradix Schenck and Smith were deposited on sterile gellan gum solidified water (0.4% Gel-Gro and 0.05% $MgSO_4.7H_2O$) amended with 0, 1, 10, 100 and 1000 ug a.i. g^{-1} of simazine, dichlobenil and paraquat in petri plates.

The herbicides were diluted in sterile water to the designated concentrations. They were either disolved, or suspended and kept under agitation, in sterile water under the laminar flow hood. One millilitre of the different concentrations of the herbicides was spread over 20 ml of solidified water. Petri plates were incubated at 28°C for 7 days. Hyphal length was then measured by the line intersect method (Tennant, 1975) under a dissecting microscope bearing a grid (10x10 1-mm squares) in the eye piece. The highest concentration of simazine and dichlobenil interfered with the measurement, and

as the mycelium could not be clearly seen, their length was not measured.

Statistical analysis

Since mycorrhizal and non-mycorrhizal plant dry mass differed, dry mass reduction was calculated as the percentage of dry mass reduction of herbicide-treated plants relative to the respective (mycorrhizal or non-mycorrhizal) non-herbicide-treated control. Similarly, we considered the relative elongation rates (i.e. elongation week ^{1} initial height ^{1}) in our analysis.

The data was subjected to analysis of variance using the general linear model (GLM) procedure of SAS (SAS Institute, 1989). The Duncan's multiple range test was used to compare treatment means and linear contrasts, to compare the average photosynthetic rates of simazine-treated plants and paraquat-treated plants to non-herbicide-treated plants (Steel and Torrie, 1980).

4.3 Results

G. intraradix colonized apple rootstocks effectively. On average, 92% of inoculated plant root systems contained mycorrhizal fungi at harvest (data not shown), while no colonization was found in non-mycorrhizal plant roots. After a lag phase of about 5 weeks, the mycorrhizal fungus increased plant growth rates (Fig. 4.1). From the sixth week on, the mycorrhizal apple rootstocks were significantly taller than non-mycorrhizal controls.

Relative plant elongation rate of mycorrhizal plants was reduced from 0.72 cm week^{-1} cm^{-1} of plant before herbicide application, to 0.17 cm week^{-1} cm^{-1} of plant four weeks after herbicide application, while that of non-mycorrhizal apple had dropped from 0.09 to 0.06 cm week^{-1} cm^{-1} of plant (data not shown). The increased susceptibility of mycorrhizal apple rootstocks was independent of the level and type of herbicide used, as indicated by the absence of an inoculation by herbicide interaction.

At harvest, 6 weeks after the application of herbicides, the adverse effect of the herbicides on plant biomass production was significant (Table 1). The reduction in mycorrhizal and non-mycorrhizal shoot dry mass, due to herbicide applications, were similar (Table 4.2). However, the reduction of mycorrhizal root dry mass in response to herbicide treatments was more pronounced than that of non-mycorrhizal roots, and plant mortality was much greater among mycorrhizal plants than non-mycorrhizal plants (Fig. 2). No control plants were killed by simazine application even at a rate of 20 ug a.i. g^{-1} soil.

On average, 23.9 mm of hyphae emerged after 7 days from spore clusters deposited on non-amended gellan gum solidified water (data not shown). Simazine did not affect hyphal growth at any level applied (Table 4.3). However, as little as 1 ug g^{-1} of dichlobenil or paraquat significantly reduced the

growth of G. intraradix in vitro. Although dichlobenil and paraquat did reduce fungal growth in vitro, they had no significant effect on the percentage of root colonization by the mycorrhizal fungus (data not shown).

Simazine and paraquat significantly reduced ($P<0.05$) the photosynthetic rates of mycorrhizal and non-mycorrhizal plants (data not shown). However, an herbicide by inoculation interaction ($P=0.018$) indicates that the fungus changed the plants response to dichlobenil. While increasing rates of dichlobenil on non-mycorrhizal plants reduced photosynthetic rates relative to the water-treated control, application on mycorrhizal plants increased it (Fig. 4.3).

4.4 Discussion

Few studies have compared the effects of herbicides on mycorrhizal and non-mycorrhizal plants. Pope and Holt (1981) found a reduction in growth and P uptake by white ash grown in soil amended with paraquat, only when the plants were mycorrhizal. The authors also measured a direct detrimental effect of 1.33 ug g^{1} of paraquat on the production of external mycelium and spores by the fungus and on colonization of ash trees, suggesting that paraquat affects plant development through its effect on the fungus.

In contrast to Pope and Holt's experiment, our work studied the effect of

herbicide treatments applied after establishment of the symbiosis. Similarly to Pope and Holt's results, we found an increase in the toxic response of P16 apple rootstocks treated with simazine, paraquat or dichlobenil when plants were mycorrhizal, but found no significant effect of the herbicides on the percentage root colonization, although the size of the mycorrhizal root systems was reduced. In addition, our in vitro germination test showed no effect due to 100 ug of simazine on G. intraradix hyphae elongation in gellified water Still, at the highest rate, 75% of simazine treated mycorrhizal plants died while there was no mortality among control plants.

Our results suggest that mycorrhizal fungi could increase plant suceptability to herbicides through improved uptake of the chemicals. This hypothesis is supported by the recent findings of Nelson and Khan (1992) who demonstrated, using compartmentalized containers and ^{14}C-labelled atrazine, that mycorrhizal hyphae were able to remove atrazine from soil and transfer it to plants. This observation could also apply to other chemicals.

To our knowledge, there is no other report on the effect of dichlobenil on mycorrhizae, but the effects of simazine and paraquat have been studied by a few researchers.

Increases in carbohydrate exudation from Chenopodium quinona, when treated

weekly with sublethal doses of simazine, was thought to be the cause of mycorrhizae formation on this otherwise non-mycorrhizal plant (Schwab et al., 1982). In a later study, Nemec and Tucker (1983) also observed that Citrus seeded in soil amended with 1.2 ug g^{1} simazine developped a significantly higher percentage of root colonization. They explained their results either by an inhibition of competing organisms or, in agreement with Schwab et al. (1982) results, an increase of root exudation.

In the same study, amending the soil with the highest level of simazine (7.1 ug g^{1}) resulted in the reduction of mycorrhizal colonization level of Citrus from 64% to 11% (Nemec and Tucker, 1983). Similarly, paraquat reduced the development of G. fasciculatum and the mycorrhizal colonization of ash trees (Pope and Holt, 1981).

Our results do not corroborate previous studies. Although dichlobenil and paraquat have the potential to harm mycorrhizal fungi as shown by the adverse effect of these herbicides on fungal growth in vitro, we did not measure any detrimental effect on root colonization of apple rootstocks following the application of dichlobenil, paraquat or simazine. We measured, however, a reduction in root growth which should have led to a reduction in the length of colonized roots and may have led to a reduction in external mycelium length. It must be emphasized also that the 6 week period between herbicide

application and plant harvest was a relatively short time for an effect to develop, and that killing a plant would result sooner or later in the reduction of the vigor for its endophyte.

The absence of a negative impact of simazine applied even at very high levels on in vitro elongating mycorrhizal hyphae is not that surprising. Although, after being taken up by roots, simazine interfers with a variety of plant biochemical processes including photosynthesis, plant growth regulation, nitrogen metabolism and nucleic acid metabolism (Esser et al. 1975), the chemical seems harmless to at least some fungi. When simazine was applied at a rate of 5 ug g^{1}, Aspergillus fumigatus was able to metabolize the herbicide as a source of carbon, while another study has shown that fungal populations of a sandy loam soil were slightly stimulated at concentrations of 2.5 to 10 ug g^{1}.

Many other herbicides were found to have no impact on mycorrhizae. Among them are tri-allate, di-allate, diuron and trifluralin on wheat (Smith et al. 1981), chlorpropham, sulfallate and phenmidian on wheat and alfalfa (Ocampo and Barea 1982), bromacil, diuron and trifluralin on Citrus (Nemec and Tucker, 1983), alachlor and trifluralin on soybean, (Burpee and Cole, 1978), oxadiazon and trifluralin on sweetgum (South, 1981). In contrast, Sieverding and Leihner (1984) observed that oxadiazon and oxifluorfen both decreased cassava root infection and spore number. Finally, Garcia-Romera and Ocampo (1988) found

variation in herbicide tolerance among species of mycorrhizal fungi.

The opposite effects of dichlobenil on the photosynthetic rates of mycorrhizal and non-mycorrhizal plants one week after application are difficult to explain. We know that dichlobenil is absorbed from the soil by the root system and translocated in the leaves (Humburg et al. 1989). Dichlobenil is a powerful inhibitor of germination and of actively dividing meristems and acts primarily on growing points and root tips. However, studies with Chlorella and Scenedesmus revealed no effects of dichlobenil on respiration or phytosynthesis (Humburg et al., 1989).

Physiological changes induced by mycorrhizal colonization could be involved in this observed interaction between colonization and dichlobenil application, as suggested by the work of Nelson and Khan (1992). They found, in their study on ^{14}C-labelled atrazine uptake by corn, that the symbiosis could change the allocation and metabolism of the herbicide, as shown by differences in the proportion of bound and methanol extractable ^{14}C-labelled s-triazine.

A better knowledge of the impact of the various herbicides used in crop production on mycorrhizae should facilitate the development of sound production strategies. This is particularly important for the management of mycorrhizae-dependent crops such as apple.

Table 4.1 Shoot and root dry mass of P16 apple rootstocks 6 weeks after herbicide application and percentage dry mass reduction relative to controls, due to herbicide treatments[z].

Herbicide treatments	Shoot		Root	
	Dry mass (g)	Reduction (%)	Dry mass (g)	Reduction (%)
Control	5.19 a	-	0.96 a	-
Dichlobenil				
1 ug g^{-1}	4.88 ab	0.05 f	0.77 ab	0.19 c
5 ug g^{-1}	3.57 bcd	0.32 cde	0.53 bc	0.43 bc
10 ug g^{-1}	2.08 f	0.56 abc	0.22 d	0.72 a
25 ug g^{-1}	1.99 f	0.63 ab	0.11 d	0.87 a
Paraquat				
0.5 ug g^{-1}	4 53 abc	0.19 def	0.61 b	0.37 c
1 ug g^{-1}	4.99 a	0.04 ef	0.75 ab	0.22 c
10 ug g^{-1}	4 79 ab	0.06 f	0.62 b	0.32 c
100 ug g^{-1}	3 43 cde	0 41 bcd	0 35 cd	0.63 ab
Simazine				
1 ug g^{-1}	2 15 ef	0.54 abc	0.27 d	0.68 a
5 ug g^{-1}	2 44 def	0.49 abc	0.30 cd	0.65 ab
10 ug g^{-1}	1.80 f	0.67 ab	0 20 d	0.76 a
20 ug g^{-1}	1.51 f	0.70 a	0.17 d	0 80 a

[z]Mean separation within columns using Duncan's multiple range test P=0 05 (n=8)

Table 4.2 Effect of mycorrhizal colonization on shoot and root dry mass of herbicide-treated P16 apple rootstocks and non-herbicide-treated controls, and on percentages of dry mass reduction 6 weeks after herbicide application[z].

Mycorrhizal status	Shoot			Root		
	Dry mass (g)			Dry mass (g)		
	Herbicide treated	Control	Reduction (%)	Herbicide treated	Control	Reduction (%)
Mycorrhizal	4.51	7.39	39 a[z]	0.43	1.21	63 a
Non-mycorrhizal	1.92	2.98	36 a	0.37	0.70	46 b

[z]Mean separation within columns using Duncan's multiple range test P = 0.05 (n = 52).

Table 4.3 Percentage of length reduction relative to non-herbicide treated controls in hyphae produced from Glomus intraradix spore clusters placed on herbicide amended gellified water after 7 days[z].

Concentrations	Herbicides		
(ug g^{-1})	Simazine	Dichlobenil	Paraquat
0	0 a	0 c	0 c
1	12 a	47 b	57 b
10	14 a	99 a	71 a
100	11 a	100 a	100 a
1000	ND	ND	100 a

[z]Mean separation within columns using Duncan's multiple range test P = 0.05 (n = 10).

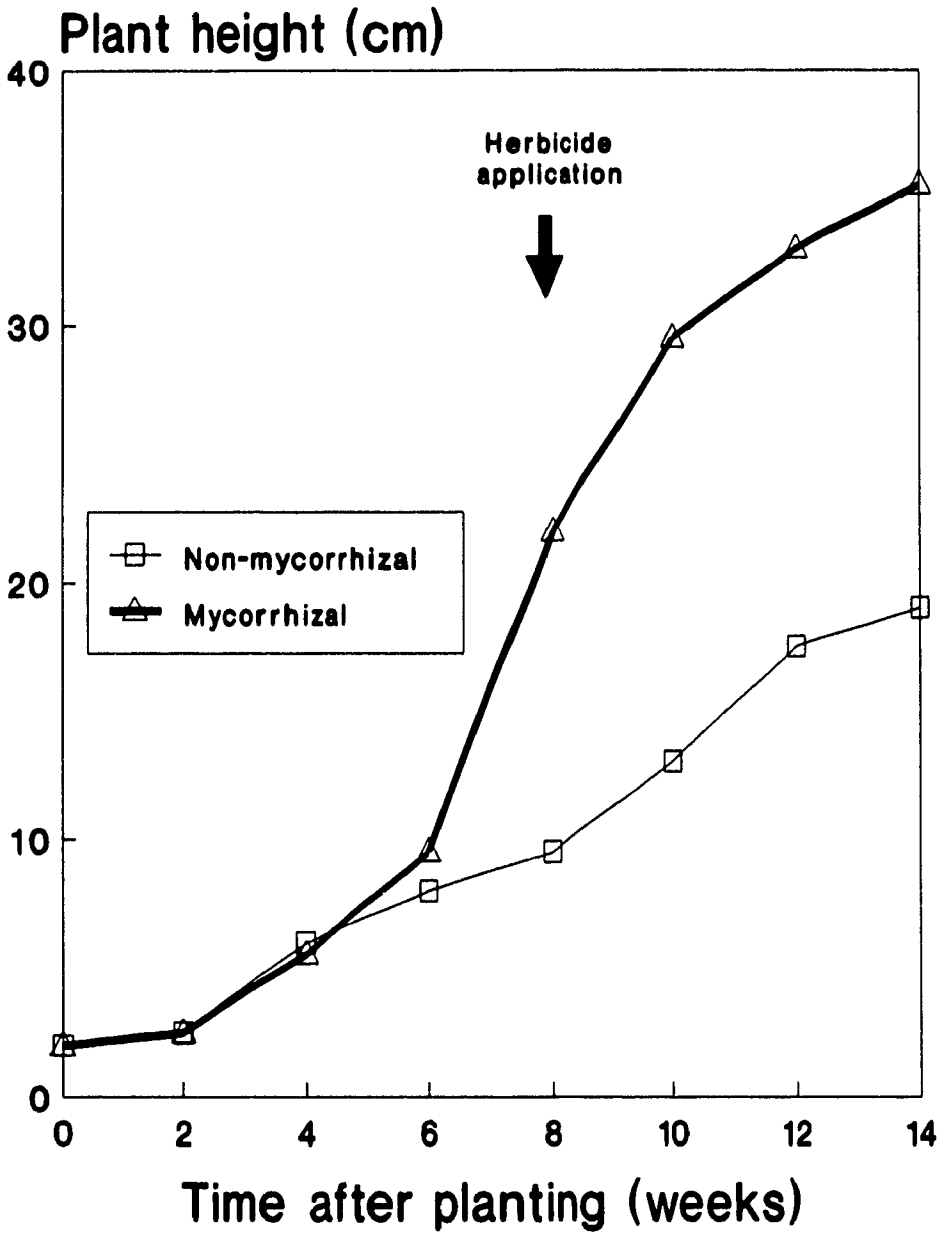

Fig. 4.1 Growth of mycorrhizal and non-mycorrhizal P16 apple rootstocks before and after herbicide treatments (four rates of simazine, dichlobenil or paraquat and a water-treated control, applied as soil drench). The arrow indicates the time of treatment application. Means ($n = 52$) are different ($P < 0.05$) from the sixth week on.

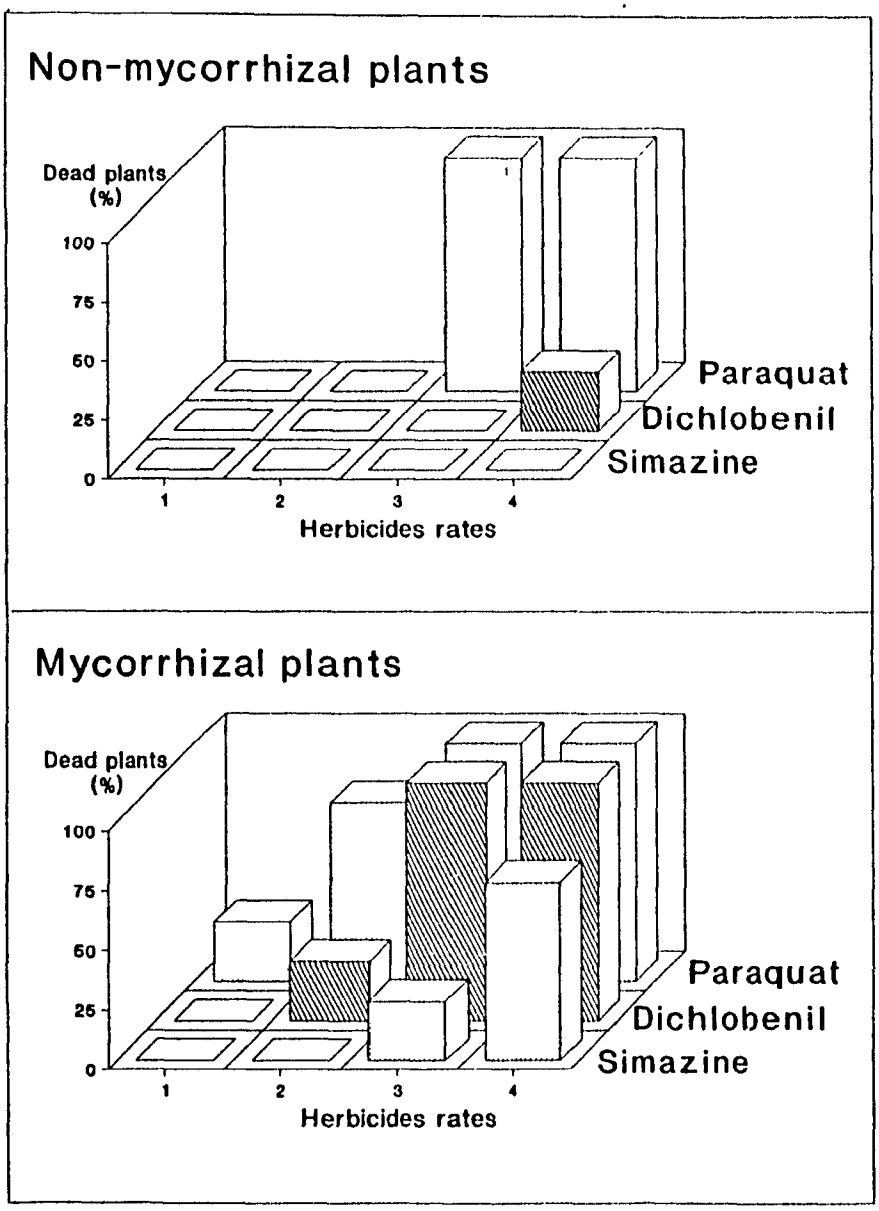

Fig. 4.2 Effect of the mycorrhizal status on P16 apple rootstock mortality 6 weeks after application of four rates of simazine (1, 2, 10 and 20 ug a.i. g^{-1}) dichlobenil (1, 5, 10 and 25 ug a.i. g^{-1}), or paraquat (0.5, 1, 10 and 100 ug a.i. g^{-1}) applied as soil drench, 8 weeks after inoculation of the plants. Bars represent means of four plants.

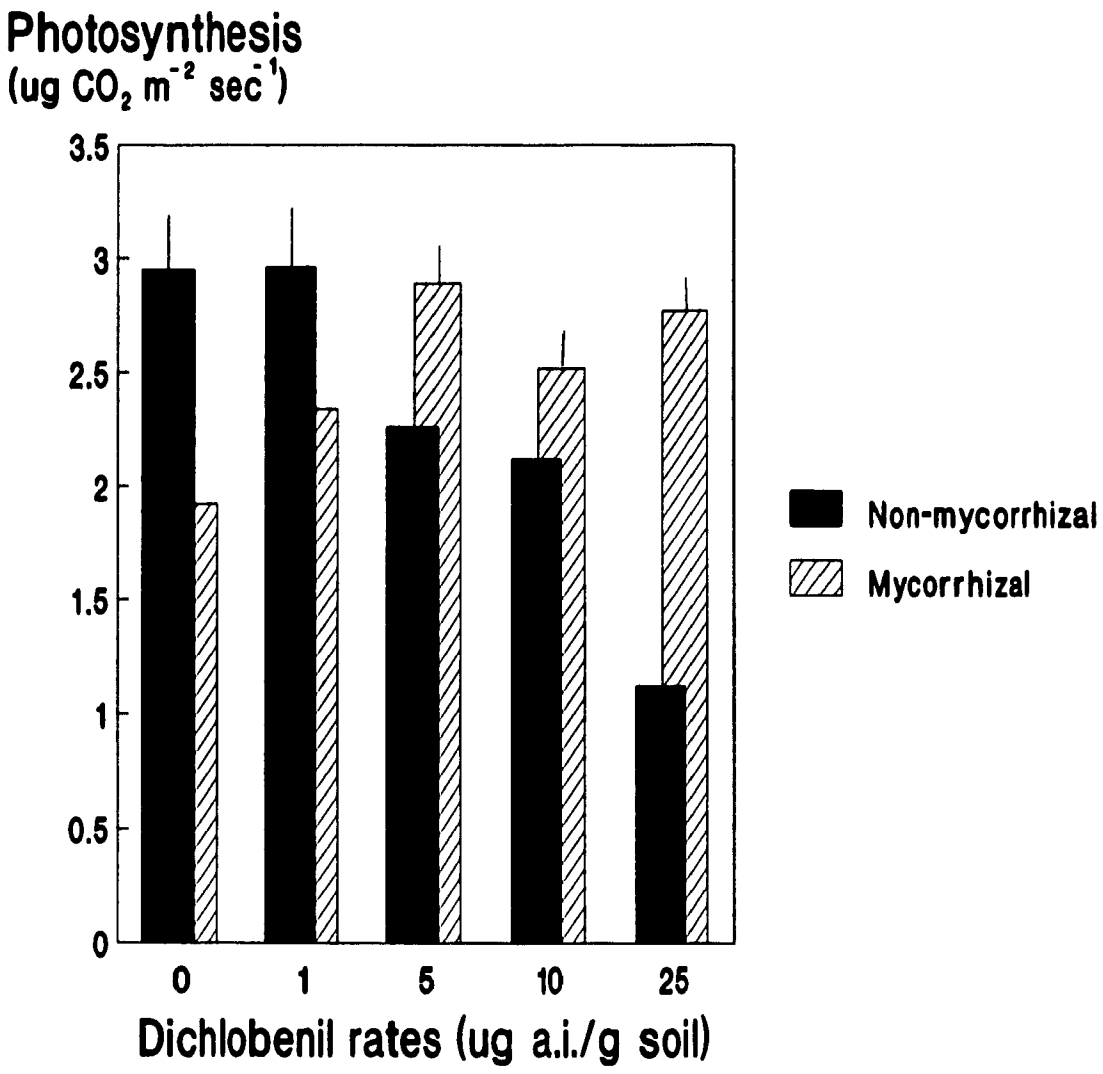

Fig. 4.3 Contrasting effects of dichlobenil application relative to water-treated controls on the photosynthetic rates of mycorrhizal and non-mycorrhizal P16 apple rootstocks. CO_2 assimilation rates were measured on the first uppermost fully expanded leaf of each plants 1 week after application of the herbicide as soil drench. Vertical bars indicate SE and $n = 4$.

Section 5

GENERAL DISCUSSION AND CONCLUSION

In orchards, and in agricultural systems in general, VA-fungi populations constitute an omnipresent resource for promoting crop production that needs to be preserved and further exploited. This can be achieved directly, through early inoculation with selected efficient species during propagation of young plants in nursery production. It can also be done indirectly, through sound use of pesticides, in order to preserve or even promote soil VA-mycorrhizal flora.

Through the research contained in this thesis, we have evaluated the efficiency of VA-fungi on apple rootstocks and acquired a better knowledge of herbicide effects on mycorrhizal apples.

5.0 Mycorrhizal effects on growth of apple rootstocks

It was demonstrated in sections 3 and 4 that apple rootstocks were very dependant on mycorrhizae. Non-mycorrhizal rootstock cultivars were at least twice as small as the mycorrhizal plants, despite the high P-levels of the soil The mineral analysis data (section 3) indicated that mycorrhizae significantly increased the plant P-levels compared to the control, while the overall nutrient

balance was also improved, as shown by the total DRIS indices. Phosphorus was the limiting element to growth of non-mycorrhizal plants, while nitrogen was limiting in mycorrhizal plants. In the colonized plants, the nitrogen deficiency was a consequence of a growth dilution effect. The data clearly show that P-deficiency was exclusive to non-mycorrhizal plants. Hence, VA-mycorrhizae improved apple growth mainly through an improved P nutrition.

Since as much as 90% of the phosphorus fertilizer applied may be rendered unavailable to plant uptake (Stevenson, 1986), VA-fungi appear particularly useful in facilitating plant P-uptake. In a low-P soil with a high fixing capacity, Covey et al., (1981) demonstrated that VA-inoculation could partially substitute for apple P-fertilization, and calculated that colonization with Glomus mosseae (Nicol. and Gerd.) Gerd. and Trappe was equivalent to a value between 400 and 800 mg kg^{1} of P fertilizer. My research showed that VA-fungi also improve growth and nutrition of apples grown in a soil with a high P-available level.

Apple rootstocks responded differently to VA species and isolates in term of leaf area production. While inoculation of P.16 and P.22 did not cause any significant effect, all VA-fungus treatments increased the leaf surface area of M.26 and Ott.3. The latter tended to respond best to G. versiforme (OR), while M.26 produced the greatest leaf surface area when inoculated with G.

versiforme (CAL).

Overall, G. versiforme (CAL) tended to be the most efficient in promoting final height, shoot dry mass, shoot/root ratio and nutrient balance. In terms of plant height, a weak interaction between cultivars and VA-fungi indicated a better response of M26 rootstocks to VA-fungi, compared to the other cultivars.

The coarse root system of apple rootstocks was very responsive to mycorrhizal inoculation, as demonstrated by the intense level of internal colonization. P.22 was less colonized than M.26 and P.16, but developed as much as the other rootstocks, in terms of plant height, dry mass production and plant nutrient balance. These results suggest that growth enhancement is not directly related to the final level of internal colonization.

While all fungi produced similar percent root colonization levels, there were differences in the extent of external hyphal network produced. The efficiency of the VA-fungi varied in relation with the extent of extramatrical hyphae, G. versiforme (CAL) producing the most extensive network. Hence, selection of VA-fungi for inoculation on apple rootstocks should consider the ability to form a large extramatrical hyphal network.

5.1 Herbicide toxicity on mycorrhizal apple

Mycorrhizae increased apple susceptibility to soil-applied herbicides (Section 4), as demonstrated by the greatly increased mortality, among mycorrhizal apples. Nelson and Khan (1992) have demonstrated that mycorrhizae could remove atrazine from soil and transfer it to host plant. In agreement with this finding, the improved absorption capacity of mycorrhizal over non-mycorrhizal roots could explain the increased herbicide toxicity on mycorrhizal plants. This hypothesis seems coherent with the greater dry mass reduction observed on mycorrhizal roots over non-mycorrhizal roots.

Both mycorrhizal and non-mycorrhizal plants had similar photosynthetic rates prior to herbicide applications (data not shown). Paraquat and simazine applications decreased the photosynthetic rate of all apple plants. However, mycorrhizae changed the plant response to dichlobenil to one very different from that observed on non-mycorrhizal plants; dichlobenil application decreased the photosynthesis by non-mycorrhizal plants, while it significantly increased the rate of mycorrhizal plants. These results suggest the involvement of physiological effects of mycorrhizae on the host plants, other than just an increased herbicide absorption.

Dichlobenil and paraquat both adversely affected fungal growth in vitro, even at the lowest rate; dichlobenil was the most toxic. Strong concentrations (100

ug g^{-1}) of paraquat and dichlobenil completely inhibited spore germination Simazine had litt.e effect on spore germination, even at a rate of 100 ug g^{1}. These results contrast with the field observations of Granger et al. (1993) where simazine residues reduced the population of spores and sporocarps of Glomus species, even one year after cessation of four annual applications.

Soil application of herbicides to established apple mycorrhizae did not significantly decrease the percent root colonization. Effects of herbicides on the extramatrical hyphae remains unknown as we have unsuccessfully attempted to quantify the vitality of these hyphae, following herbicide application.

5.2 Conclusion

In the future, research should be conducted to allow a better understanding of the impact of herbicides on the physiology of mycorrhizal associations. This presupposes a thorough comprehension of the physiological effects of the fungus over the host plant, which is to be achieved.

Studies of herbicides effects on mycorrhizal plants are particularly important for the management of crops such as apple, which are both extremely dependant on mycorrhizae and rely greatly on herbicide applications for weed management. Hence, improved tree performance gained through early inoculation of apple plants may be partially altered by herbicide sprayings. In

this regard, our results should be verified and quantified under field conditions, with standard application methods, in order to select herbicides that control weeds without adversely altering mycorrhizal fungi and mycorrhizal plants. The knowledge of herbicide effects on mycorrhizal apples is important for the development of sound integrated crop management programs.

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