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The effects of arbuscular mycorrhizal colonization by *Glomus intraradices* on the susceptibility of maize (*Zea mays* L.) to damage caused by the Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte)

by
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A thesis submitted to the Faculty of Graduate and Postdoctoral Studies,
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ABSTRACT

The effect of arbuscular mycorrhizal colonization by *Glomus intraradices* on the susceptibility of maize, *Zea mays* L., to damage caused by a major North American corn pest, the western corn rootworm larvae, *Diabrotica virgifera virgifera* LeConte, was assessed under greenhouse and laboratory conditions. This is one of the few studies evaluating the potential effect of arbuscular mycorrhizae on macroscopic soil-borne pests.

Two separate greenhouse trials (1998 & 1999) were done in order to compare the performance of the maize hybrid Pioneer 3921 under western corn rootworm feeding stress with and without mycorrhizal association. In the 1998 greenhouse experiment, we observed a significant increase in standard plant-growth parameters such as plant height, stalk circumference, and total mass in mycorrhizal maize compared to the non-mycorrhizal controls. We also observed a reduction in the average root damage ratings on the Iowa 1-6 scale of plants associated with *Glomus intraradices*. The mycorrhizal effect in this greenhouse trial was greater in plants submitted to lower infestation rates (200 eggs/plant) compared to the highest infestation rate (1000 egg/plant). In the 1999 greenhouse experiment, emerging western corn rootworm adults from the mycorrhizal treatments showed reductions in head capsule diameter. The differences in head capsule diameters were greatest at the intermediate infestation rates (150 eggs/plant).

The biochemical profiles of maize roots grown for 2 and 4 weeks under greenhouse conditions were determined using HPLC to test for the possible mycorrhizal induction of the major hydroxamic acid found in maize, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-(4H)-

one (DIMBOA) and its degradation product 6-methoxybenzoxazolinone (MBOA). A persistent trend of higher DIMBOA equivalent concentrations in mycorrhizal roots compared to those from the control plants was observed. This trend however was not statistically significant at the accepted critical p-value ($\alpha = 0.1$). In roots of plants harvested at 2 weeks of age, a significantly higher concentration of MBOA was observed in the mycorrhizal treatment compared to the non-mycorrhizal control.

RÉSUMÉ

Nous avons évalué l'effet des mycorhizes à arbuscules de l'espèce *Glomus intraradices* sur la susceptibilité des plants de maïs, *Zea mays* L. à un des principaux déprédateurs du maïs en Amérique du Nord, la chrysomèle des racines du maïs de l'ouest, *Diabrotica virgifera virgifera* Le Conte. Tous les résultats obtenus proviennent d'expériences qui ont été effectuées en serre et en laboratoire. Cette étude est une des rares études servant à démontrer un effet potentiel des mycorhizes à arbuscules sur l'interaction entre les plantes cultivées et leurs pestes macroscopiques édaphiques.

Deux expériences en serre (1998 et 1999) ont été effectuées afin d'évaluer la performance des plants de l'hybride du maïs Pioneer 3921 soumis au stress produit par les larves de chrysomèles du maïs de l'ouest et en association avec le champignon mycorhizien *Glomus intraradices*. Des plants non-mycorhizés ont été aussi observés pour fin de comparaison. Lors de l'expérience de 1998, nous avons obtenu une augmentation significative au niveau des paramètres standards de croissance du maïs mycorhizé par rapport au maïs non-mycorhizé. Ces paramètres comprennent la hauteur, la circonférence des tiges et la biomasse des plants de *Z. mays*. C'était aussi chez le maïs mycorhizé que nous avons observé la plus grande réduction du dommage larvaire aux racines selon l'index de dommage racinaire Iowa 1-6. L'effet mycorhize sur le développement du maïs était à son maximum pour les plantes assujetties au taux d'infestation le plus bas, soit 200 oeufs/plantes. Nous avons aussi observé, lors de l'expérience de 1999, une réduction de la taille des capsules céphaliques des adultes émergeant des systèmes racinaires avec mycorhizes. Les plus grandes différences au niveau des diamètres de capsules céphaliques se sont manifestées aux taux d'infestations moyens (150 oeufs/plante en 1999).

Les profiles biochimiques des racines de maïs cultivé pour 2 et 4 semaines sous des conditions de serres ont été déterminés par CHPL afin de tester la possibilité d'induction par colonisation mycorhizenne de l'acide hydroxamique majeure du maïs, soit le 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-(4H)-one (DIMBOA), et le produit de sa dégradation, le 6-methoxybenzoxazolinone (MBOA). La concentration du DIMBOA avait tendance à être plus élevée dans les racines de maïs mycorhizés. Cette tendance n'est, par contre, pas statistiquement significative. A deux semaines de croissance, une concentration significativement plus élevée de MBOA a été observée dans les racines mycorhizées par rapport au racines non-mycorhizées.

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CHAPTER I

GENERAL INTRODUCTION

1.1. Economic importance of maize

Surpassed only by wheat and rice, maize is the third largest crop in the world. Total world area harvested for maize in 2000 was evaluated at over 138 million Ha with a total production of almost 600 million Mt. (FAO 2001). The top two maize producing nations in the world, at the present time, are the United States and China with a production of over 253 million and 105 million Mt respectively (FAO 2001). Canada is presently the 11th largest maize producer. The total Canadian maize production was evaluated at over 9 million metric tons with a total cultivated area of 1178 kha for the 2000 growing season (Agriculture and Agri-Food Canada 2001). Maize is Ontario's most important crop plant. In 1998, the maize industry contributed over $67 million to the Quebec and Ontario economy. The global importance of maize both from an economic and nutritional stand point cannot be overstated.

The maize plant is without a doubt one of the earliest and most important examples of the genetic modification of a crop by humans. Because of its complete domestication, maize is entirely dependant on humans for its seed dispersal. Similarly to other crops such as beans, squash, chili and tomatoes, the cultivation of maize finds its origins in Mesoamerica. It is commonly held that maize arose from the domestication of a group of Mexican wild grasses called teosinte. The specific variety of teosinte, *Zea mays parviglumis,* is presently believed to be the direct ancestor of our cultivated maize (Doebley 1990).

1.2. The maize plant

1.2.1. General description

Zea mays ssp. *mays*, a monocot, is a member of the Poaceae, or grass family. As other plants found in warmer climates, maize benefits from the C4 photosynthetic pathway. Even though its origins are tropical, various hybrids of this annual cereal crop are readily cultivated in subtropical and temperate regions of the world. Maize is an exceptionally large plant compared to other members of the Poaceae. It is characterised by its rigid upright stalk along which alternate narrow leaves are arranged on opposing sides. Further characteristics of the plant include conspicuous nodes and predominant brace roots arising from the aerial nodes close to the ground. Maize is monoecious whereby the female and male flowers are separate but present on the same plant. The male inflorescence, or the tassel, is borne on the stem apex whereas the female inflorescence, the ear, develops at the apex of lateral branches protruding directly from a node.

Maize relies on wind pollination for sexual reproduction. Fertilization of the ovaries leads to the development of caryopses, single dry fruits typical of the Poaceae. These maize grains are more commonly referred to as kernels.

The development of the maize root system begins at the radicle, or embryonic root, stage. A transverse section of a typical maturing maize root reveals the various tissues composed of different cell types. The outer most cell layer is the epidermis which gives rise to the relatively short lived root hairs. These structures significantly increase the absorption surface of the roots. Around the inner circumference of the epidermis lies the cortex, the largest component of the monocot root. This tissue plays an important role in the transport of substances via the numerous plasmodesmata interconnecting the cortical cells. It is also of importance to the plant resistance to root-bound pathogens. It has been shown that the greatest accumulation of defensive metabolites, such as DIMBOA (Xie et al 1991) is found in the these cortical cells.

The lateral roots are produced close to the apical meristem and extend from the pericycle which is a thin layer of cells located between the root cortex and the vascular tissues (the phloem and xylem). The morphology of the maize root system is characterized by nodes, structures created by the distinct growth of roots in an annular arrangement. The number of nodes range from 7 to 10 in typical maize varieties.

The variations between the different types of maize most widely grown in modern agriculture depend principally on traits associated with the grains' endosperm, the starchy reserves comprising approximately 85% of the grains dry weight. Five major types of maize are commonly recognized and clearly illustrate the changes in grain characteristics brought upon by years of breeding by farmers (Salvador 1997). Flint corn, which presently accounts for about 14% of global maize production, has small, characteristically hard grains and is mostly used as livestock feed and for industrial purposes. One of the older types, the starchy Flour corn is usually cultivated for direct consumption by growers and composes approximately 12% of global maize production. It is this type of maize used to make the traditional corn flatbread known as tortillas. Dent corn derives its name from the characteristic indentation of each kernel caused by the loss of moisture from the starchy crown of the kernel and the subsequent reduction of the endosperm volume. This type of corn is widely used both as livestock feed as well as a major component of industrial manufacturing of foods such as syrup, starch, oils and alcohol. It is presently the most prevalent type of maize, constituting around 73% of the world wide commercial maize production. Sweet corn, with its high sugar content is generally used fresh, canned or frozen for human consumption. However, despite its popularity in the North American market, sweet corn accounts for less than 1% of global maize production. Pop corn, the type of maize best known as the snack food of the same name, has a small spherical kernel containing an endosperm with a very high moisture content and a notably hard pericarp. The production of this type of maize also accounts for less than 1% of global production.

Maize growth and development is favoured by warm temperatures. Very little if any growth occurs at temperatures lower than 10°C, thereby increasing the time for the plant to attain maturity. The daytime temperature optimal for growth of corn is estimated at 30°C (Bonhomme 1984). Temperatures exceeding this optimum will also reduce crop development rates. Other environmental factors affecting maize development include photoperiod, moisture and soil fertility.

The maize hybrid used in this study is Pioneer 3921®. This is a relatively rapid growing hybrid requiring approximately 2600 crop heat units (CHU) to reach physiological maturity. Most commercial maize hybrids are rated within the range of ca. 2100 to almost 3500 CHU. CHU's reflect the accumulated daily heat units for a given growing season. Daily heat units take into account both day time air temperature highs and night time lows. The use of such units are specially useful in estimating the time required for maturity of crop plants due to their highly temperature-dependant rates of development (Brown and Bootsma 1993).

1.2.2. Major insect pests of maize crops

Maize is subject to attack by a wide array of phytophagous insect pests. Every part of the plant is susceptible to pest damage at different stages of the maize life cycle. Some of the more economically important leaf-feeding pests include the armyworms (*Spodoptera spp.*) and the corn leaf aphid (*Rhopalosiphum maidis*).

Stem damage caused by insect attack results in serious economic loss every year in maize growing regions. The European corn borer (*Ostrinia nubilalis*) is one of the most damaging and widely distributed of the stem boring pests. Extensive damage to maize crops was recorded in Ontario and Québec as early as the mid 1920's (Parks 1926).

Other pests such as the corn earworm (*Helicoverpa zea*) bore into the young ears causing serious damage to the developing grain. The importance of post harvest loss to grain pests should not be understated. Major stored grain pests to maize include the Angoumois grain moth (*Sitotroga cerealella*), the maize weevil (*Sitophilus zeamais*), and the large grain borer (*Prostephanus truncatus*).

The current efforts focused on reducing the negative impacts of insect damage to economically important crops have in large part revolved around integrated pest management (IPM). IPM arose in response to the serious problems associated with synthetic pesticides such as DDT and 2-4-D which were heavily relied upon as pest management tools in the 1950's and 1960's and remain important elements of present control strategies. IPM is an approach aimed at establishing environmentally sound pest control strategies that rely on plant resistance, biological control agents and cultural practices all the while limiting input of synthetic pesticide and other sources of potentially damaging pollutants (Burn et al 1987). Furthermore, with the prices for maize steadily decreasing since 1994, a record production year (Nafziger and Bullock 1999), it is increasingly important to discover and adopt new agricultural practices, including pest control strategies, that require lesser inputs of costly synthetic chemicals thereby reducing production costs to the growers.

It has been estimated that American maize producers lose over 1B$ US to a group of insect pests known as the Corn Rootworm complex (Metcalf 1986). These economic losses are due in part to crop damage as well as pest-control cost. The Corn Rootworm complex consists of four economically important species of diabroticite rootworms which are part of a large genus of the galerucine chrysomelids in the order Coleoptera.

The Mexican corn rootworm (*Diabrotica virgifera zeae*), originating from central America has a distribution range limited mostly to eastern Mexico and central Texas. It is a serious pest to continuous maize in these regions (Stewart 1999). The southern corn rootworm (SCR) (*Diabrotica undecimpunctata howardii* Barber) is also known as the spotted cucumber beetle because of the distinctive 12 black markings on the adult beetle's elytra. The SCR is considered to be a minor maize pest in most of North America. Extensive damage to maize crops in Texas and Louisiana however have been reported and has caused important economic loss in these areas (Drees 1999).

The northern corn rootworm (NCR) (*Diabrotica barberi* Smith and Lawrence) a pest native to North America was first reported in Colorado (Tollefson and Levine 1999). Its North American distribution spreads from the Atlantic coast of the continent westward all the way to the Rocky Mountains and from southern Ontario southward to Tennessee. As a result, in great part, of the practice of continuous corn planting, the present NCR range has greatly expanded since the middle of the 20th century when it was mostly limited to the north-central American Corn Belt (Tollefson & Levine 1999).

The fourth species of the corn rootworm pest complex to be listed here will be the subject of our research and experiments. This is the Western Corn Rootworm (WCR), *Diabrotica virgifera virgifera* LeConte.

1.3. *Diabrotica virgifera virgifera*

1.3.1. Geographic distribution

The Western corn rootworm, which often co-occurs in maize fields with the NCR, is the most economically important member of the CR complex in Canadian maize production. It was originally described in Nebraska by LeConte in 1868. The first report of maize damage caused by this insect occurred in 1909 in the states of Colorado and Kansas. Before the mid-1990's, their range was mostly limited to the eastern slope of the Rocky Mountains from New Mexico to Nebraska. However, due in great part to the increasing use of continuous corn cultivation, an easterly extension of the WCR range has been observed in the second half of the twentieth century. The rate of range extension during this period has been evaluated at 64-80 km/year, finally reaching the North American Atlantic cost in the middle of the 1980's. The present day WCR range in North America is almost continent-wide. The only parts of the corn-growing region in which they are absent is west of the Rocky Mountains and into the southeastern parts of the United States.

1.3.2. Description and life cycle of the Western Corn Rootworm

The western corn rootworm is a univoltine species. Egg hatch occurs in mid to late spring. The larvae, which feed almost exclusively on maize roots, go through three larval instars before pupation in early summer. Adults emerge from the soil and begin copulation soon after. Ovipositing occurs ca. 14 days later when the adult females deposit the eggs on soil surface in late summer. A female western corn rootworm will live on average 80 days and has a lifetime fecundity that can reach more than 1000 eggs (Branson and Johnson 1973). The western corn rootworm overwinter as eggs in diapause. The typical western corn rootworm life cycle is summarized in figure 1.1.

1.3.3. Current WCR control methods

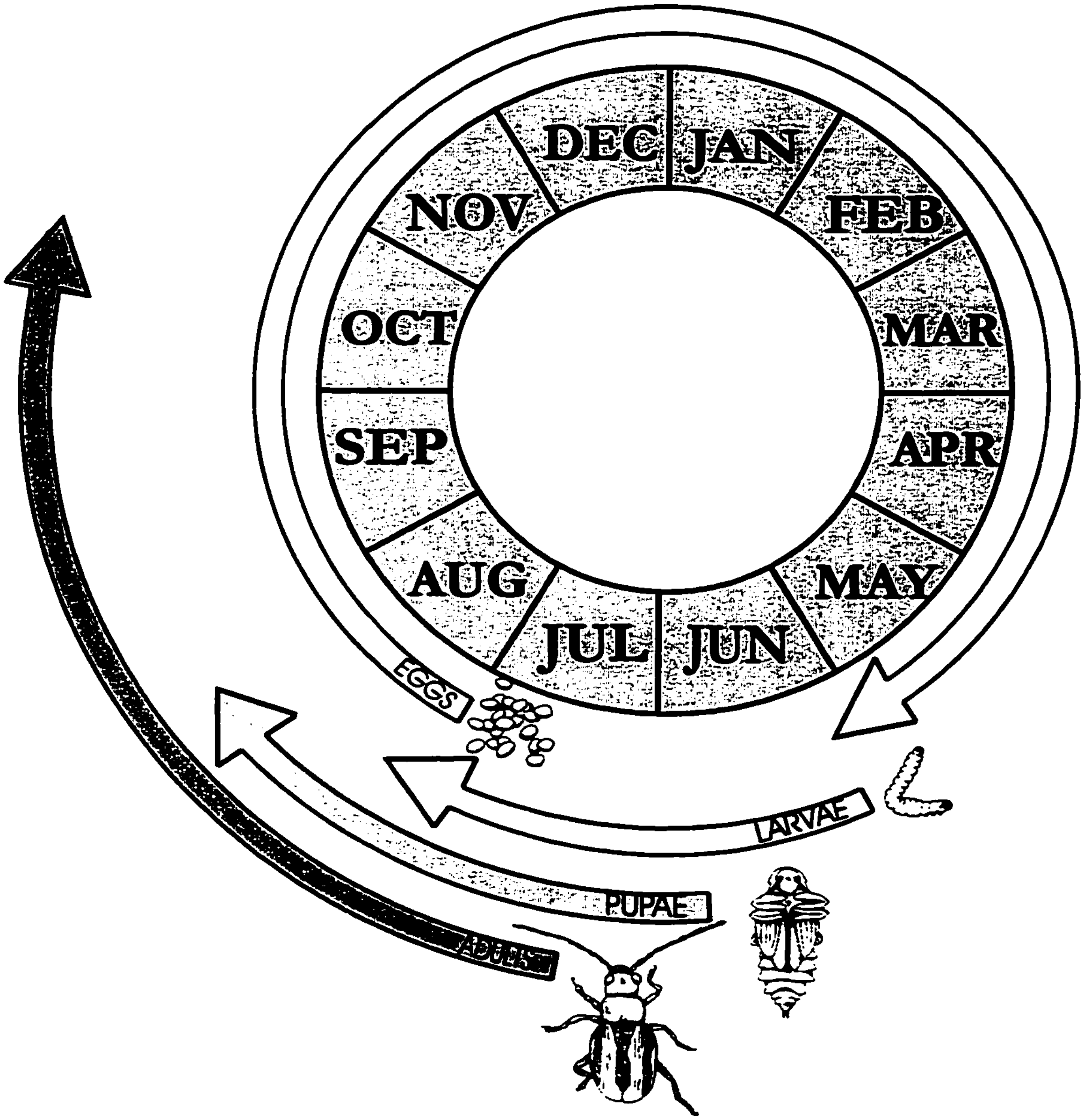
The application of synthetic insecticides to control corn rootworms is still quite prevalent in continuous maize cultivation despite environmental and health concerns. Chemical control strategies of corn rootworm damage have focussed on protecting maize root systems through the application of soil insecticides (Levine and Oloumi-Sadeghi 1991). An estimated 25% of the total North American corn growing area is treated at the time of planting with soil-applied insecticides (Sutter 1999). This number increases to 90% when taking into account only the areas where maize is grown continuously.

1.3.4. Non-pesticide WCR-control strategies

Because of the western corn rootworm's univoltine nature and high specificity to maize as host plant, crop rotation has been thus far the most efficient non-chemical management method (Levine & Oloumi-Sadeghi 1991). Since eggs are laid in maize fields by the adult females, the larvae are unable to attain maturity when the maize plants are replaced by alternative crops in the subsequent growing season.

Figure 1.1:

Life cycle of *Diabrotica virgifera virgifera*, the western corn rootworm


DEC
JAN
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AUG
SEP
OCT
NOV
EGGS
LARVAE
PUPAE
ADULTS

However, the reduction of crop rotation efficiency as a rootworm control method is a potentially serious problem. Recently, severe WCR damage to maize crops rotated with soybean *Glycine max* L. has been recorded in Illinois and northwestern Indiana (Levine and Oloumi-Sadeghi1996). Two probable causes have been suggested to explain the development of CR resistance to crop rotation. The first of these is the occurrence of a prolonged diapause over two winters instead of the ususal one (Levine et al. 1992). The pest is then able to avoid the stress of emerging in the unsuitable environment that is the alternative crop. Nonetheless, this type of modification in the CR life cycle has only been observed in NCR eggs and very rarely in WCR (Levine et al. 1992).

Alternatively, adult female WCR have recently been reported to oviposit in soybean fields, the crop most commonly used for rotation with maize in non-continuous systems. This modified behaviour seems to have emerged in a new strain or biotype of the WCR (Sammons et al. 1997). The prevalence of such a strain could potentially eliminate the efficiency of maize rotation with soybean entirely.

So far, strategies for western corn rootworm resistance have been focused on the development of maize varieties with increased root masses and a greater ability of recovery after larval feeding damage (Reidell 1993, Branson 1989). Examples of biological control methods, such as applications of parasitic nematodes (Jackson 1996) are few and have shown limited potential.

1.4. Maize defence and resistance

Plant resistance to phytophagous insects generally falls under one of the three categories proposed by Painter (1951). These are **tolerance, antixenosis** and **antibiosis.**

Tolerance by the plant, is achieved through various mechanisms allowing the endurance of or recovery from stress related to insect-caused injury. Antixenosis, originally referred to as non-preference (Kogan and Ortman 1978), results from a plant's unattractive qualities and/or its unsuitability as a potential target for phytophagous insects. On the other hand, the plant attains antibiosis through resistance factors that have direct negative impacts on the invading pathogens or herbivores.

These resistance factors are often found under the form of secondary metabolites produced within the different plant tissues (Swain 1977). These compounds are referred to as secondary because of their apparent lack of role in plant primary metabolism. Due to this apparent absence in plant primary metabolism, secondary metabolites were originally thought to be waste products (Fraenkel 1959, Muller 1969).

Many members of the Poaceae family, including maize, wheat and rye are known to produce various hydroxamic acids. The principal hydroxamic acid found in maize, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-(4H)-one (DIMBOA), has been identified as an important resistance factor against various phytophagous insects such as *Ostrinia nubilalis*, *Helicoverpa zea*, *Diabrotica* spp. and multiple species of aphids. Resistance to pathogenic fungi and bacteria has also been shown to increase through DIMBOA action (Niemeyer 1988).

DIMBOA is found as a glucoside in uninjured maize tissue in the roots, stalk and leaves. Upon injury, a hydrolytic enzyme, glucosidase, is released resulting in the accumulation of the aglycone form of DIMBOA. It is readily degraded into its main degradation products, the benzoxazolinones MBOA (6-methoxybenzoxazolinone) and formic acid. The biosynthesis and degradation of DIMBOA are presented in figure 1.2. Xie et al.

(1992) suggested that MBOA may also have a negative effect on the WCR larvae. It does not, however, seem to occur naturally in living tissues of the maize plant (Niemeyer 1988).

DIMBOA concentrations are highest in root tissue (Klun and Robinson 1969) and generally tend to decrease with increasing age of the plant. Hydroxamic acid levels have been shown to increase with damage of plant tissue at early developmental stages (Morse et al. 1991). These observations reveal a probable defence response on the part of the plant through hydroxamic acid action.

1.5. Mycorrhizal symbiosis

1.5.1. The origin of mycorrhizae

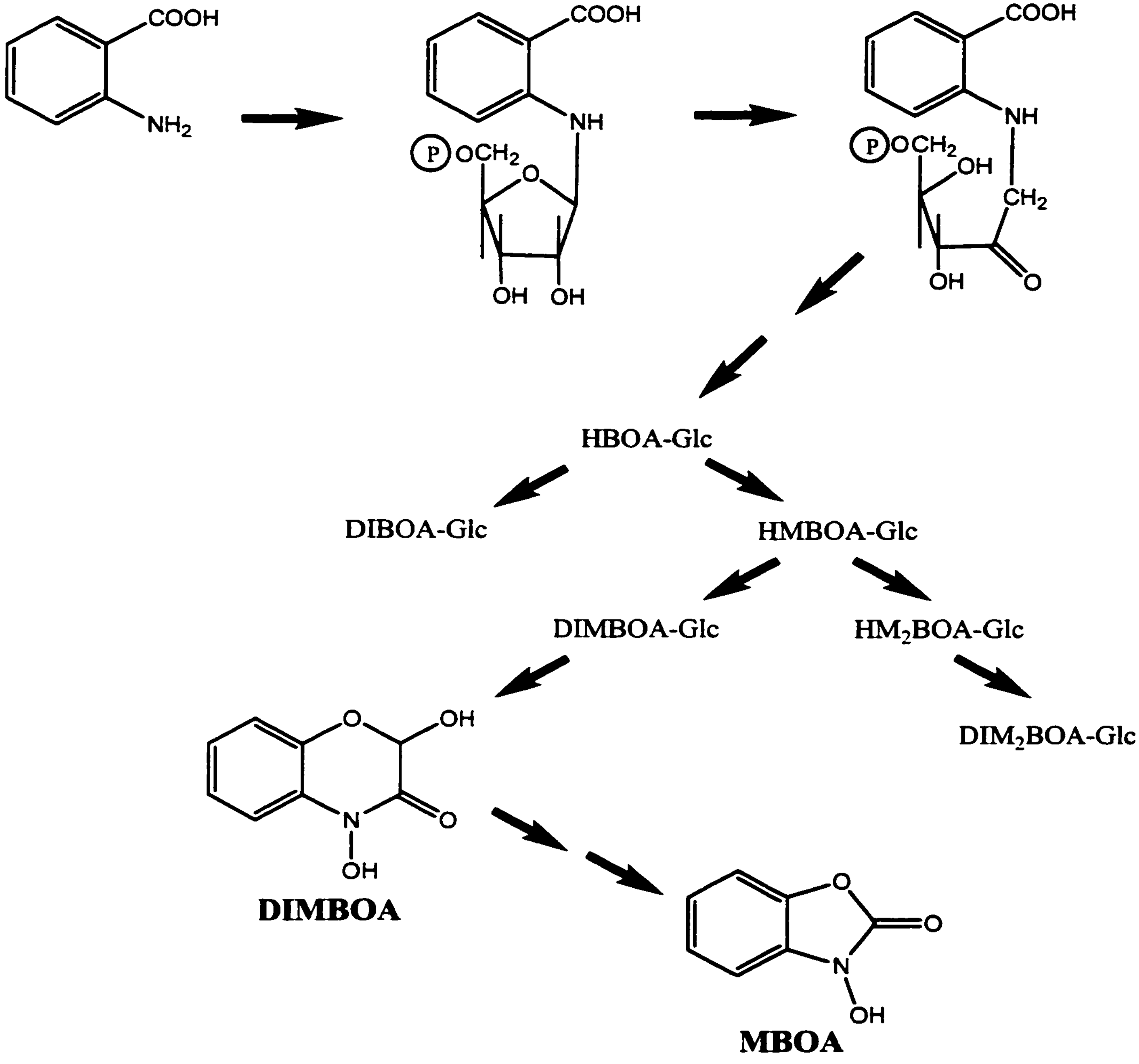
Mycorrhizae are believed to have arisen over 400 million years ago (Stubblefield et al. 1987; Pirozynski & Dalpé 1989; Simon et al. 1993). It is widely held that mycorrhizal associations may have in fact played an important role in the terrestrial invasion of plants (Pirosynski and Malloch 1975). In order to mediate the harsh terrestrial conditions likely prevalent during early land colonization, it is thought that modifications occurred to the pre-existing relationship between plants and parasitic fungi (Allen 1991), which gave rise to new beneficial plant-fungus associations.

The term mycorrhiza, introduced by Frank (1885), describes the mutualistic symbiosis existing between a fungal partner and what is most often the roots of higher plants, i.e., the Gymnosperms and Angiosperms. Some of the earlier plants such as pteridophytes and bryophytes have also been observed to form symbiotic relations between their underground gametophytic organs and fungi. The mycorrhizal association is quite widespread in natural environments. Most higher plants are believed to readily form

Figure 1.2:

Biosynthesis and degradation of DIMBOA and other hydroxamic acids

(adapted from Niemeyer 1988)


COOH
NH2
COOH
NH
P OCH2
O
OH OH
COOH
NH
P OCH2
OH
CH2
OH
O
HBOA-Glc
DIBOA-Glc
HMBOA-Glc
DIMBOA-Glc
HM2BOA-Glc
DIM2BOA-Glc
O
OH
N
O
OH
DIMBOA
O
O
N
OH
MBOA

beneficial associations with various mycorrhizal species. Estimates have indicated that over 90-95% of all terrestrial plants are mycorrhizal at some stage of their life cycle (Allen 1991).

There are a few plant families, however, such as the Chenopodiaceae and the Brassicaceae, that tend to be non-mycotrophic and do not normally enter this type of mutualistic partnership. Nonetheless, under experimental conditions, limited mycorrhizal potential has been demonstrated even in these plants that are traditionally considered as non-mycorrhizal (Allen 1983, Tommerup 1984, Glenn et al. 1985). Various types of mycorrhizal associations are recognized and differ on the basis of structure as well as host specificity. Some mycorrhizae are very specific in regards to host plant family and in certain cases can even be genus- and species-specific. Ericoid and orchid mycorrhizae are found exclusively in association with roots of plants from the Ericaceae and Orchidaceae families respectively. Other types of mycorrhizae, however, are ubiquitous in regards to their choice of host, as will be discussed further. One of the two most widespread types of mycorrhizae in nature are the ectomycorrhizae, which are easily recognizable by their characteristic hyphal arrangement, the Hartig net, that forms an extracellular network in the root cortex of the host plants. The second type, and most widely distributed mycorrhizae, are the arbuscular mycorrhizae. These were originally referred to as the endomycorrhizae.

1.5.2. Arbuscular Mycorrhizae

Contrary to the ectomycorrhizae, arbuscular mycorrhizae (AM) are characterized by a fungal penetration through the walls of the host plant's root cortical cell. Within these cells, the AM fungus forms characteristic structures specific to this type of symbiosis. Arbuscles, from which the mycorrhizae's name is derived, are tree-shaped structures formed by intracellular hyphal extensions and serve as the exchange site between the two symbiotic partners.

The term *vesicular arbuscular mycorrhizae* (VAM), although commonly used to describe this type of mycorrhizal symbiosis, is not accurate. Vesicles are characteristic structures found in AM whose principal role is that of a reserve organ. Although they are found in most of the AM associations, their presence is not obligatory and is not always observed. Besides their importance for lipid storage, vesicles appear also to be efficient mycorrhizal propagule (Smith and Read 1997).

A network of extracellular and characteristically aseptate hyphae extend outside of the roots and into the soil, thus considerably augmenting the interface area between the host plant and its growth substrate. This is the first proposed mechanism explaining increases in nutrient and water uptake associated with mycorrhizal colonization. A second apparent mechanism depends on the mycorrhizal fungus' ability to gain access to nutrient sources which otherwise would be unavailable to the plant. Due to the much smaller diameter of the hyphae compared to that of plant roots, AM are able to penetrate in small pores of soil particles giving access to a larger pool of ions with low mobility such as P, Zn and Cu (Linderman 1992).

In exchange, the fungal element of the AM mutualism draws benefits from the autotrophic nature of the host plant by integrating complex carbohydrates through the arbuscular exchange sites. Fitter (1991) estimated the total sugar cost to the host plant for maintaining the AM fungal association at approximately 10%.

1.5.3. Arbuscular mycorrhizal diversity

Host plant selection by the arbuscular mycorrhizal fungus is a highly non-specific affair. This is clearly illustrated when considering that although overall mycorrhizal colonization of higher plants has been estimated at over 95% in certain ecosystems (Allen 1991), only 151 species of AM fungus have been identified thus far. All of the arbuscular mycorrhizal fungi are members of the order Glomales in the phylum Zygomycota. To date, only five genera are known to form arbuscular mycorrhizae; *Acaulospora*, *Entrophospora*, *Gigaspora*, *Scutellospora* and *Glomus*. *Glomus intraradices* will be the mycorrhizal species used as the experimental subject in this work.

1.5.4. Establishment of a functional association between roots and mycorrhizal fungus

The establishment of a functional mycorrhizal symbiosis between plant roots and fungus includes six important steps (Bonfante-Fasolo & Spanu 1992). These are *attraction*, *adhesion*, *penetration*, *plant reaction*, *interface establishment*, and *metabolite exchange*. The colonization process begins with spore germination. Many different factors have been suggested to either stimulate or inhibit the germination of mycorrhizal spores (Hepper and Mosse 1975, Hepper and Smith 1976, Hepper 1979). Among the factors found to be inhibitory to germination are high concentrations of heavy metals (Zn, Cd and Mn), organic acids, various sugars (see Smith and Read 1997) and high salinity (Juniper and Abbott 1993). Conversely, extracts and exudates from various host plants have been shown, in some cases, to have stimulatory effects on mycorrhizal spore germination (Graham 1982, Gianinazzi-Pearson et al. 1989).

Stimulatory effects on hyphal growth have been demonstrated in the presence of flavonoids commonly found to play key roles in other plant symbiotic associations. One specific flavonol, quercetin, has repeatedly been shown to stimulate mycorrhizal hyphal growth and branching (Gianinazzi-Pearson et al. 1989, Tsai and Phillips, 1991. Bécard et al 1992) CO_2 is another known stimulant to hyphal extension. There also appears to be a synergistic effect of flavonoids on hyphal growth stimulation by CO_2 (Bécard and Piché, 1989).

Once the hyphae extending from the germinating spore has adhered to the root, an enlargement, or *appressoria*, is formed in response to the fungal recognition of its potential host (Giovannetti et al 1993). Colonising hyphae will then project from the appressoria into the root cell wall. The mycorrhizal hyphae does not, however, breach the host cell membrane at any time during the symbiotic cycle. The dichotomous branching of the colonizing hyphal extension leads to the characteristic formation of the arbuscule.

The enveloping of the arbuscule by the host cell membrane will lead to the development of an apoplastic compartment. An interfacial matrix then forms between the fungal cell wall and the host membrane. This matrix consists of structural molecules similar to those found in plant primary cell walls such as β (1,4) glucans, non-esterified polygalacturonans and hydroxyproline-rich glycoproteins. However, unlike those found in primary cell walls, these molecules are not polymerized in the interfacial matrix (Smith & Read 1997)

The arbuscular structures are relatively short-lived with cycles varying with different fungus and plant species involved in AM. The total cycle length for the fast growing crop species *Triticum aestivum* was evaluated to be 7 days, 2-3 of which were used for arbuscule formation (Brundett et al. 1985).

1.5.5. Plant response to fungal infection

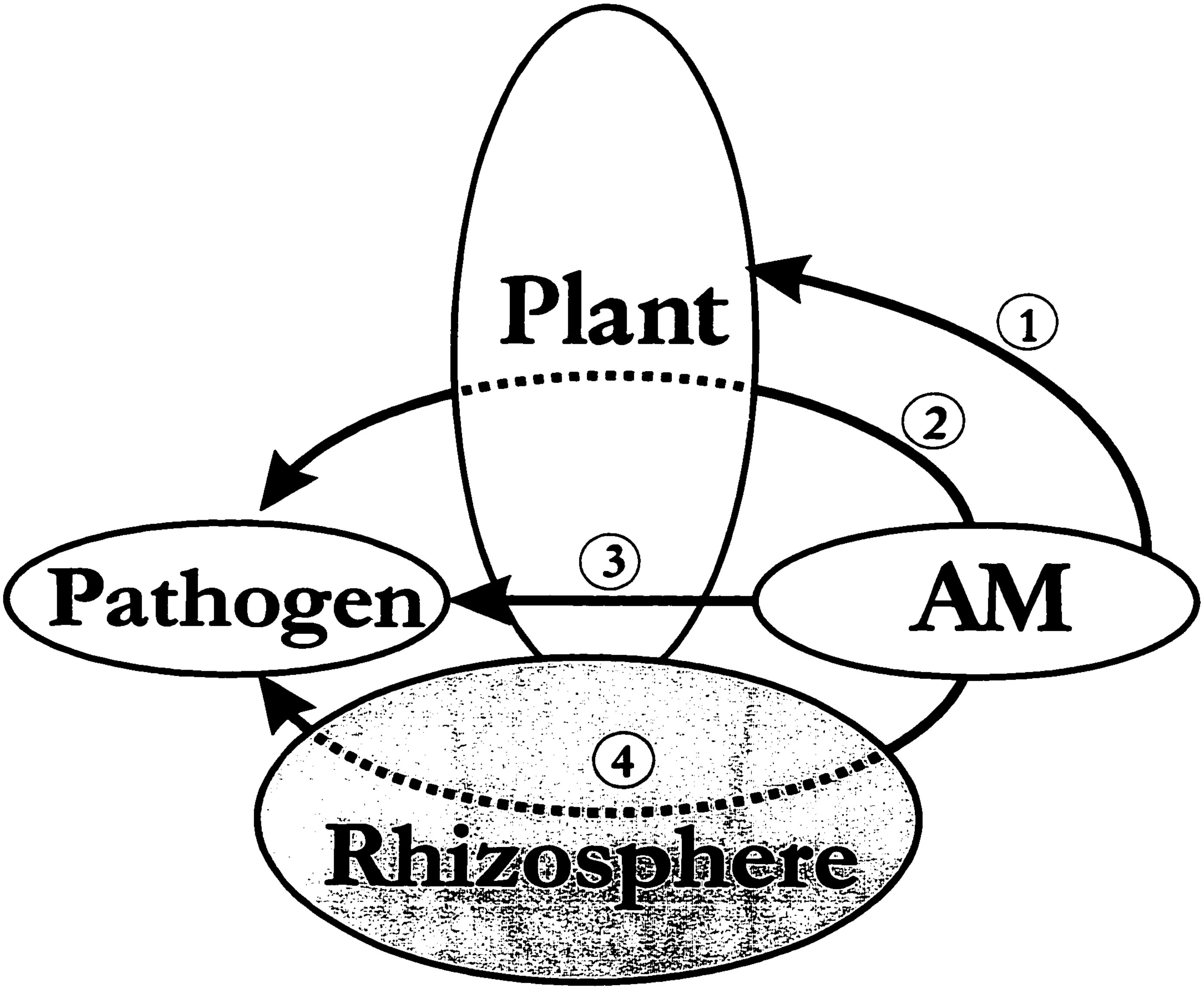
Plants may exhibit a wide range of responses to invading fungal organisms. These responses are often similar in parasitic and mycorrhizal colonization (Bonfante-Fasolo & Spanu 1992). Included among these is the production of cell wall modifying compounds such as cell wall bound phenolics, lignin and cell wall peroxidases (Koide and Schreiner 1992). Of special interest to plant fungal resistance is the induction of the hydrolitic enzyme chitinase. Findings support a mechanism by which the penetrating endomycorrhizal fungus are able to counter this type of plant response. This is accomplished through the production of as of yet undetermined soluble cell wall components that confer protection against enzymatic degradation of the fungal chitin (Spanu et al 1989, Bonfante-Fasolo et al 1990)

Numerous studies have demonstrated increases of stress resistance in mycorrhizal plants to various types of root pathogens, including fungus, nematodes, viruses and bacteria. Four major types of mechanisms (see figure 1.3) behind mycorrhizal induced resistance to pathogens in plants have been proposed (St-Arnaud et al. 1995): 1) Increases in the nutritional and water status of plants whereby losses caused by pathogen damage are compensated by the mutualstic association. 2) The stimulation of a host plant defense response by colonizing fungi has been observed, suggesting a potential mechanism behind mycorrhizal induced resistance. These host plant responses include increased chitinase synthesis (Dehne et al. 1978), stimulation of phenol metabolism and lignification in mycorrhizal roots (Dehne & Shonbeck 1979) 3) Direct deleterious effects of mycorrhizal fungus on root pathogens has often been suggested (Caron et al. 1985 & 1986a,b, Garcia-Garrido & Ocampo 1989) but has yet to be clearly demonstrated. Such demonstrations are extremely difficult due to the obligate symbiotic nature of the arbuscular mycorrhizal fungus

Figure 1.3:

Proposed mechanisms behind AM-related resistance to pathogens.

1) Better nutritional and water status, 2) induced host plant defense, 3) direct AM effect on pathogen and 4) indirect AM effect on pathogen via rhizosphere interactions and the resulting inability of isolating fungus-pathogen interactions. 4) The last mechanism proposed is the indirect effect of arbuscular mycorrhizae on root pathogens through the


Plant
①
②
Pathogen
③
AM
④
Rhizosphere

modification of interactions between pathogens and the biotic components of the rhizosphere. For example, these modifications can come under the form of changes in soil bacterial composition in the area adjacent to mycorrhizal hyphae (Meyer & Linderman 1986).

1.5.6. Arbuscular mycorrhizae and agrosystems

In order to maximize mycorrhizal effects in agricultural systems, two strategies may be adopted. The first approach is the direct application of endogenous mycorrhizal inoculum. The alternative is a manipulation of indigenous AM populations. Various agronomic practices have been shown to impact mycorrhizal effectiveness as well as propagule density (Sieverding 1991). These include fertilization, pesticide application as well as cultural practices such as crop rotation and intercropping. However, the potentially interacting effects of all these different practices in high input systems such as agricultural fields often make it difficult to know whether the impacts are going to be positive or negative. Sieverding (1991) did, however, show a clear negative effect on indigenous mycorrhizal populations due to applications of general biocides such as methyl bromide and metam-sodium.

The many benefits of the establishment of functional mycorrhizal associations in crops include increases in yield, faster relative growth rates and higher survival rates of plants subjected to various stressors. These benefits combined with reductions in long term cultivation costs could translate into higher earnings for producers.

1.6. Hypothesis

The underlying hypotheses of this study is that VA mycorrhizae formed between the endomycorrhizal species, *Glomus intraradices*, and the maize hybrid Pioneer 3921 will have the effect of reducing maize susceptibility to WCR and the inherent damage caused by larvae. In addition to the extended hyphal network's ability to compensate for damaged root mass, the occurrence of *cross protection* (Larcher 1995) against larval attack and fungal colonization is possible because of the similarities between early-stage mycorrhizal colonization and pathogenic fungal infection. Moreover, a hypothetical mechanism is proposed to explain this potential mycorrhizal effect on maize plants. The possibility that mycorrhizae have an inducive effect on maize root secondary metabolite accumulation, specifically, the WCR resistance factor DIMBOA will be tested.

1.7. Objectives

1-The putative mycorrhizal induction of plant resistance factors to pathogens has been extensively studied. However, these studies have focussed principally on pathogens of fungal, viral and bacterial nature. The relation between roots and parasitic nematodes have also been the subject of many studies. The understanding of the tri-trophic interactions between the mycorrhizal fungi, the plant roots and insect pathogens, however, is still very limited. The main objective of this work is to investigate the possible role of VA mycorrhizae in maize resistance to a macroscopic invertebrate pathogen such as the Western Corn Rootworm.

2- The second objective of this work is to test mycorhizae as potentially useful elements in IPM strategies aimed at controlling WCR related damage to maize crops.

3-And finally, this research aims to underline the importance of including consideration for arbuscular mycorrhizal associations in the establishment of agricultural practices for maize cultivation.

CHAPTER II

Effects of the Arbuscular Mycorhizal fungus, *Glomus intraradices*, on Maize Performance Under Western Corn Rootworm Damage Stress

2.1. Introduction

The arbuscular mycorrhizal (AM) symbiosis has long been recognized as being mutualistic (whereby both partners derive benefits from each other). The fungal element in this type of association is an obligate symbiont and must be associated with the host plant roots in order to develop and reproduce. The fungus clearly benefits from this association with the host plant by using it as a readily available source of carbohydrates.

Among the better understood benefits of AM to the host plants are increased nutrient and water uptake through the hyphal network. The beneficial AM effects on plants, however, are sometimes apparent only when growth occurs under unfavourable conditions (Subramanian and Charest 1995). This suggests the possibility of increased resistance to environmental stresses in plants associated with AM fungi.

Numerous studies have previously shown that mycorrhizal association increases the resistance of various host plants to abiotic stresses such as drought (Subramanian and Charest 1995 & 1997), cold (Paradis et al., 1995), and soil infertility (Jeffries 1987). Further investigations have also shown similar mycorrhizal effects in plants subjected to biotic stresses such as fungal (Bodker et al. 1998; Cordier et al. 1998), viral and bacterial pathogens (see St-Arnaud et al., 1995). Few studies, however, have focussed on mycorrhizal plant response to stress imposed by macro-invertebrate pests. This present experiment was conducted in order to test the effects of mycorrhizal colonization by *Glomus intraradices* on maize susceptibility to Western Corn Rootworm (WCR) stress in a greenhouse environment. The Western Corn Rootworm, *Diabrotica virgifera virgifera* is one of the most damaging pests of North American maize crops. Economic loss associated with the damage and management of the different Corn Rootworm species has been evaluated at over 1B$ U.S. (Metcalf 1986). Current infestations require that more than 25% of maize acreage be treated with insecticides at planting time (Sutter 1999).

In order to determine maize response to the mycorrhizae, a series of standard plant growth parameters were analysed. The adult WCR were also examined at time of emergence to evaluate the possible impact of the mycorrhizal association on the insect itself.

The hypotheses underlying this experiment are that 1) the mycorrhizal fungus *Glomus intrardices* will have a beneficial effect on maize resistance to WCR damage, and that 2) the mycorrhizae will have a negative effect on WCR development. If these hypotheses are to be supported the following should be observed: an increase of plant growth parameters and a decrease in root damage in the mycorrhizal plants compared to the non-mycorrhizal maize. Furthermore, adult WCR emerging from pots in the mycorrhizal treatment should show reduced development compared to those in the control treatment.

2.2. Material and methods

2.2.1. Plant material and growth condition

Three seeds of the commercial maize hybrid Pioneer 3921 were planted in 6L round plastic pots. After germination, each pot was thinned to 1 plant / pot. Ten plants per treatment were used in this experiment. The growth substrate consisted of equal parts peat moss, top soil and perlite. The substrate was mixed and sterilised before planting. The light regime was 14h:10h (light:dark) under natural lighting (~800 W/m^2 at midday). The plants were placed in a completely randomized block with 10 plants / treatment.

The maize plants were fertilized with a high nitrogen and low phosphorous water-soluble fertilizer: Plant Food® 30-10-10. Every week, 500ml of the fertilizer solution, at a concentration of 2ml of fertilizer mix / litre of water, was applied to every pot.

2.2.2. Mycorrhizal inoculation

The mycorrhizal fungus, *Glomus intraradices* was applied as a soil-based inoculum obtained from Premiertech®. In half of the pots, 250 ml of the inoculum containing propagules of *G. intraradices* was applied at time of planting. To establish a non-mycorrhizal control treatement, 250 ml of the same inoculum without any mycorrhizal propagules was added to the sterilized growth substrate. At 12 weeks, 70 root segments per treatment were stained and used to evaluate mycorrhizal colonization using the colonized root segment percentage method modified from Dalpé (1993). Any roots showing the presence of either hyphae, vesicles and/or arbuscles were considered colonized.

2.2.3. Root staining

The roots were washed in distilled water and then boiled in 2.5% KOH for 5 minutes. The roots were then rinsed with distilled water and acidified by soaking overnight in 1% (v/v) HCl. The roots were heated for 5 minutes in a 0.05% aniline blue solution, cooled to room temperature and rinsed in a destaining solution (3X). Fine root segments (1cm long) were cut and placed on slides for microscope observation.

2.2.4. Artificial infestation

The WCR eggs were obtained in a state of diapause from French Agricultural Research in Lamberton, MN. The eggs were washed from the sandy storage substrate with distilled water and a No.60 U.S. standard sieve (0.25mm mesh opening). The eggs were then suspended in a 0.125% agar solution. The number of eggs per ml of solution was then adjusted at 200 eggs/ml and 50 eggs/ml in 1998 and 1999 respectively. Artificial infestation was done at time of planting by applying the desired amount of infestation solution with a 1ml Gilson® Pipetman in 0.5 cm deep trenches in the soil directly above the planted seeds. In 1998, the eggs were applied at rates of 200, 600 and 1000 eggs/pot. The rates applied in 1999 were 50, 100 and 200 eggs/pot. The infestation rates in the 1999 trial were reduced for reasons discussed later. Viability was assessed by placing a determined number of WCR eggs (~50) in petri dishes lined with filter paper. These were placed in an incubator at a temperature of 25C. Hatching larvae were counted and removed every second day. A total of 240 and 425 eggs were used in 1998 and 1999 respectively for the viability tests.

2.2.5. Emerging adult capture

Traps were set up around the plant stalks at 6 weeks after planting to capture the emerging adults. The traps were made of 40 cm^2 pieces of fine white nylon mesh. A foam collar was sewn to the centre of the trap to permit lateral stalk growth (figure 2.1). The traps were attached to the growing plants by sewing the collar around the stalks and placing an elastic band around the pots in which the edges were tucked in, sealing off the area inside each pot. The trapped adults were collected every second day then immediately frozen, weighed and measured under a dissection microscope.

2.2.6. Parameters measured

The following standard plant parameters were measured at maturity (12 weeks after planting): plant height, root and shoot dry masses and stalk circumference. Root damage was also assessed using the Iowa 1-6 root damage index (Hills & Peters, 1971), where a value of 1 was given to plants with no visible WCR damage and 6 was given to plants with 3 nodes completely pruned.

In order to assess WCR development, the weights and head capsule diameters of individual emerging adults were also measured.

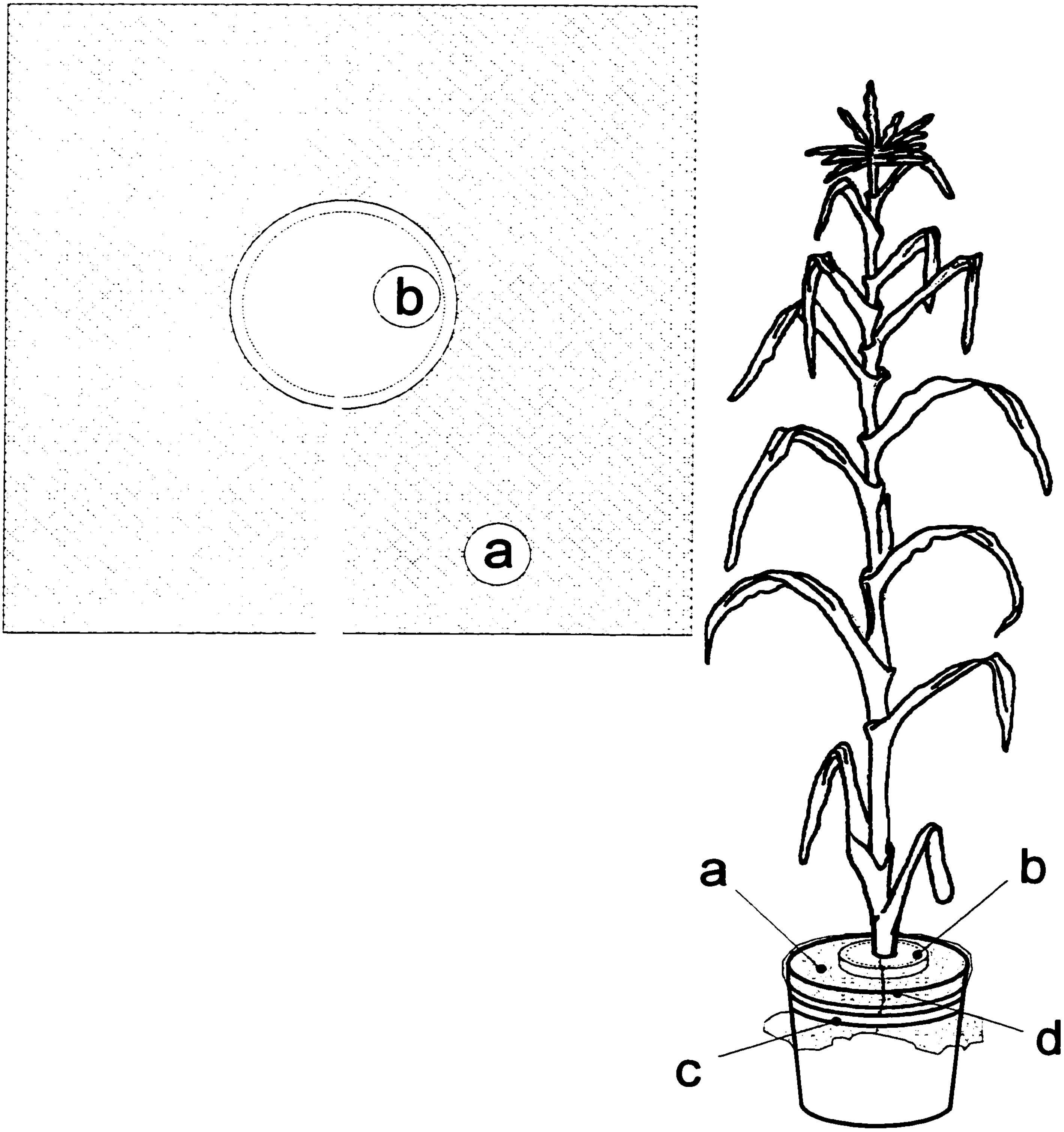
2.2.7. Statistical analyses

The data were tested for heteroscedasticity and normality. All the plant and WCR growth parameters were analysed using a parametric two-way ANOVA on the non-transformed data except for the root damage index where a non-parametric two-way

Figure 2.1

Diagram of traps used to capture emerging adults.

a- 40 cm^2 of white nylon mesh; **b**- white foam collar; **c**- elastic band; **d**- plastic pot


b
a
a
b
d
c

ANOVA was used. Bonferonni's pairwise comparisons were used when significant overall effects were observed.

2.3. Results and Discussion

Mycorrhizal colonization was 21% in the 1998 trial and 36% in the 1999 trial. No mycorrhizae were observed in any of the control treatments. The WCR egg viability was evaluated at 67.9% and 44.0% in the 1998 and 1999 trials respectively.

The results from the 1998 trials show significant mycorrhizal effects on all of the plant parameters measured (Table 2.1, Figs 2.2-2.6). In the 1999 trial, a significant effect was observed where the mycorrhizal maize plants showed decreased root damage ratings compared to the control plants. There was also a marginally significant increase of root mass in mycorrhizal maize in the 1999 trial. These results support the hypothesis that mycorrhizae are able to reduce susceptibility to WCR stress. A large portion of the losses associated with WCR damage is caused by lodging of maize plants. Because of this, the increased root dry mass observed in the mycorrhizal treatment is a potentially important factor in maize resistance to larval damage under field conditions.

Furthermore, the impact of even a slight reduction in root damage due to mycorrhizal association can be very significant. The root rating threshold for economic damage was originally accepted at 2.5 to 3.0 on the Iowa 1-6 scale (Turpin et al. 1972, Mayo 1986). It is believed by some that this threshold is actually too low and that many maize crops,

Table 2.1 Summary of mycorrhizal and infestation effects on rootworm infested maize plants (see appendix for full ANOVA tables). MYC = mycorrhizal treatment; WCR = infestation rates; M*W = mycorrhizal and infestation interaction

Variables	p-values (1998)			p-values (1999)		
	MYC	WCR	M*W	MYC	WCR	M*W
Height	**	ns	**	ns	ns	ns
Stalk circumference	***	**	*	ns	ns	ns
Root dry mass	**	**	**	0.11	*	ns
Shoot dry mass	*	**	**	ns	ns	ns
Root damage	**	ns	ns	**	***	ns

Statistical significance compared to controls: * $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$; ns: $p > 0.1$

Figure 2.2

Effect of mycorrhizal colonization by *G. intraradices* on root dry mass of artificially infested maize plants at maturity in1998 greenhouse trial. The error bars represent the standard error of the mean.

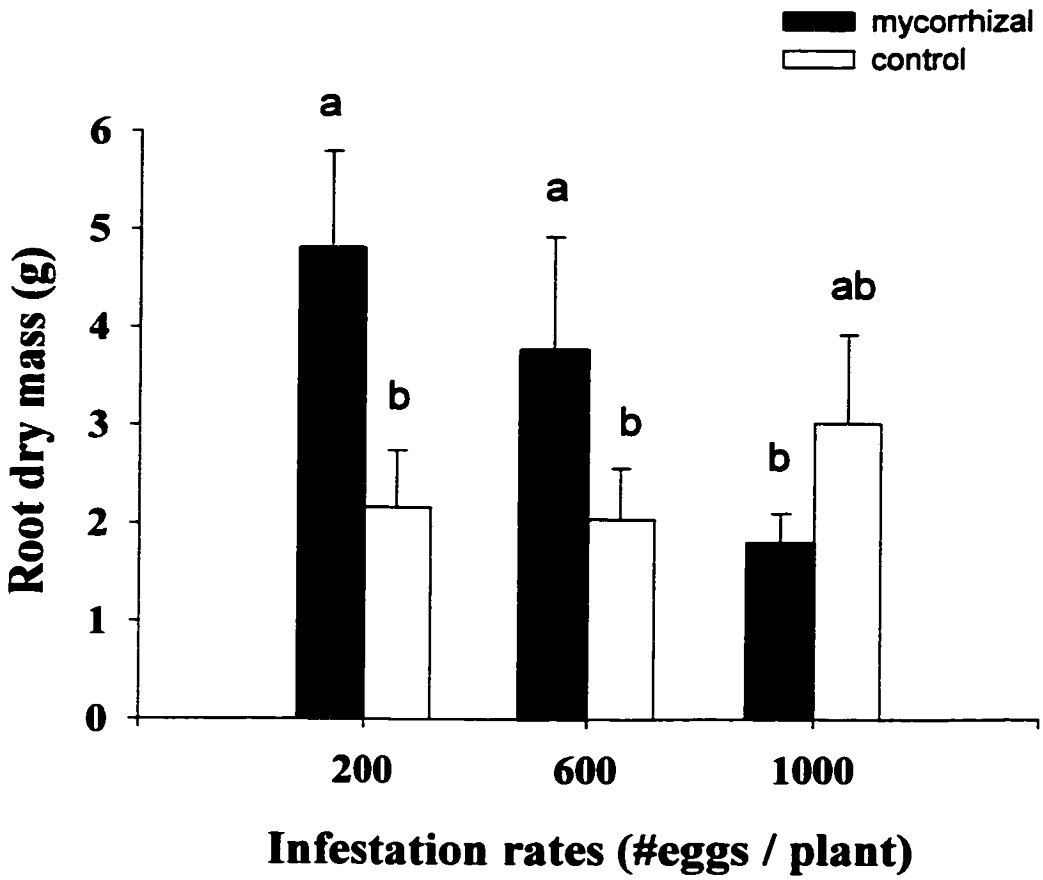

mycorrhizal
control
Root dry mass (g)
6
5
4
3
2
1
0
a
b
a
b
b
ab
200
600
1000
Infestation rates (#eggs / plant)

Figure 2.3

Effect of mycorrhizal colonization by *G. intraradices* on shoot dry mass of artificially infested maize plants at maturity (1998).The error bars represent the standard error of the mean.

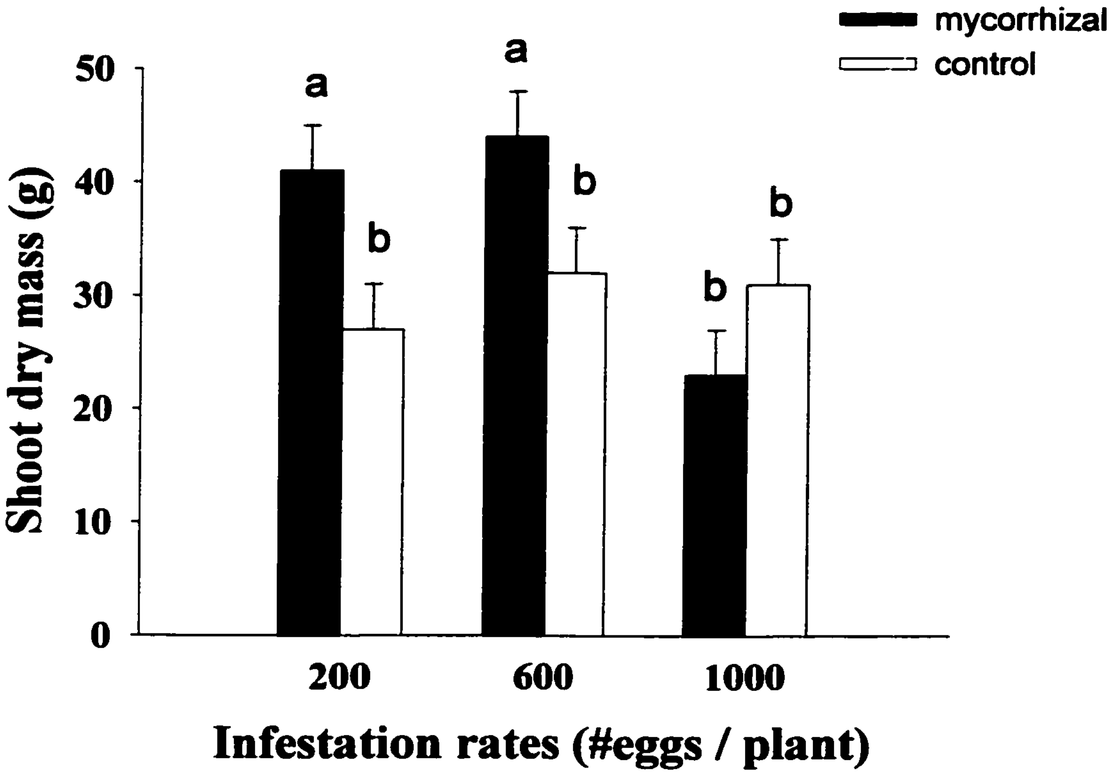

mycorrhizal
control
Shoot dry mass (g)
50
40
30
20
10
0
a
b
a
b
b
b
200
600
1000
Infestation rates (#eggs / plant)

Figure 2.4

Effect of mycorrhizal colonization by *G. intraradices* on height of artificially infested maize plants at maturity(1998). The error bars represent the standard error of the mean.

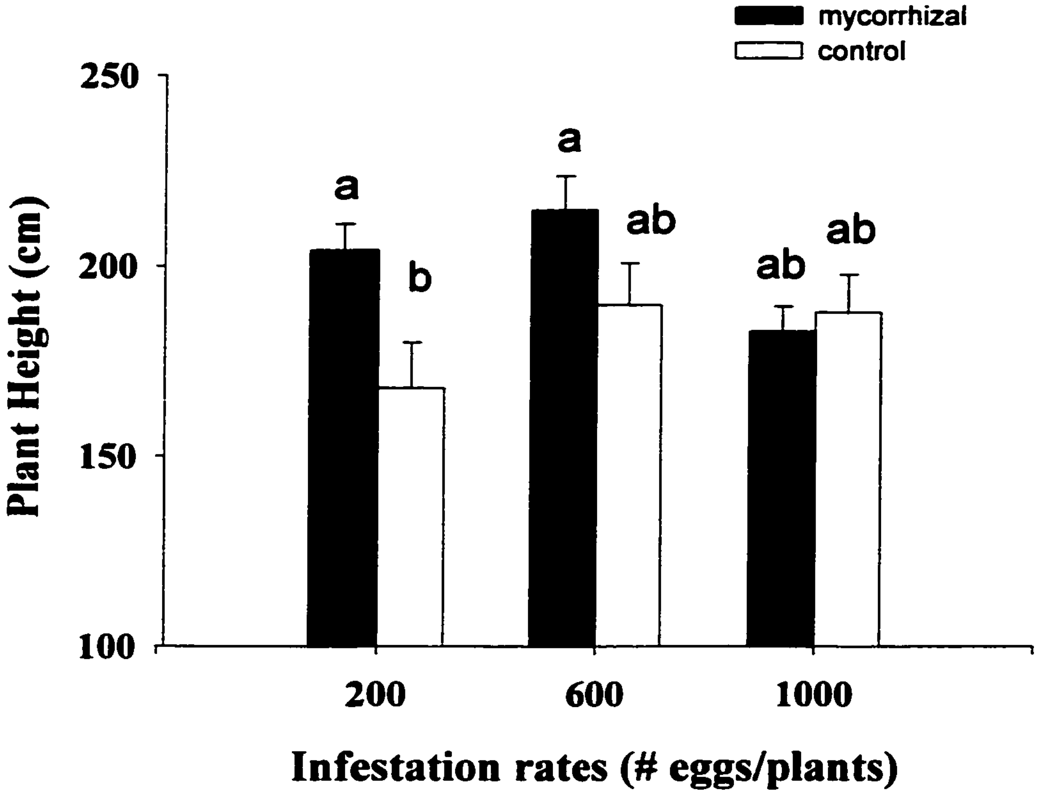

mycorrhizal
control
Plant Height (cm)
250
200
150
100
a
b
a
ab
ab
ab
200
600
1000
Infestation rates (# eggs/plants)

Figure 2.5

Effect of mycorrhizal colonization by *G. intraradices* on stalk circumference of artificially infested maize plants at maturity (1998). The error bars represent the standard error of the mean.

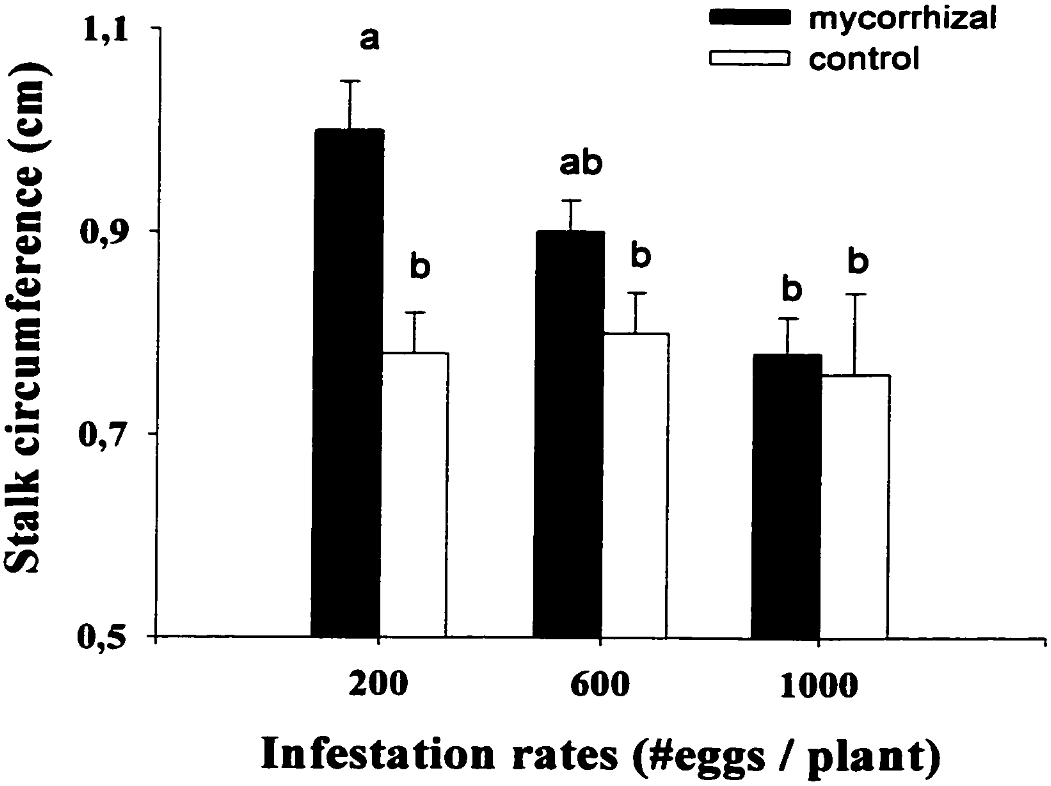
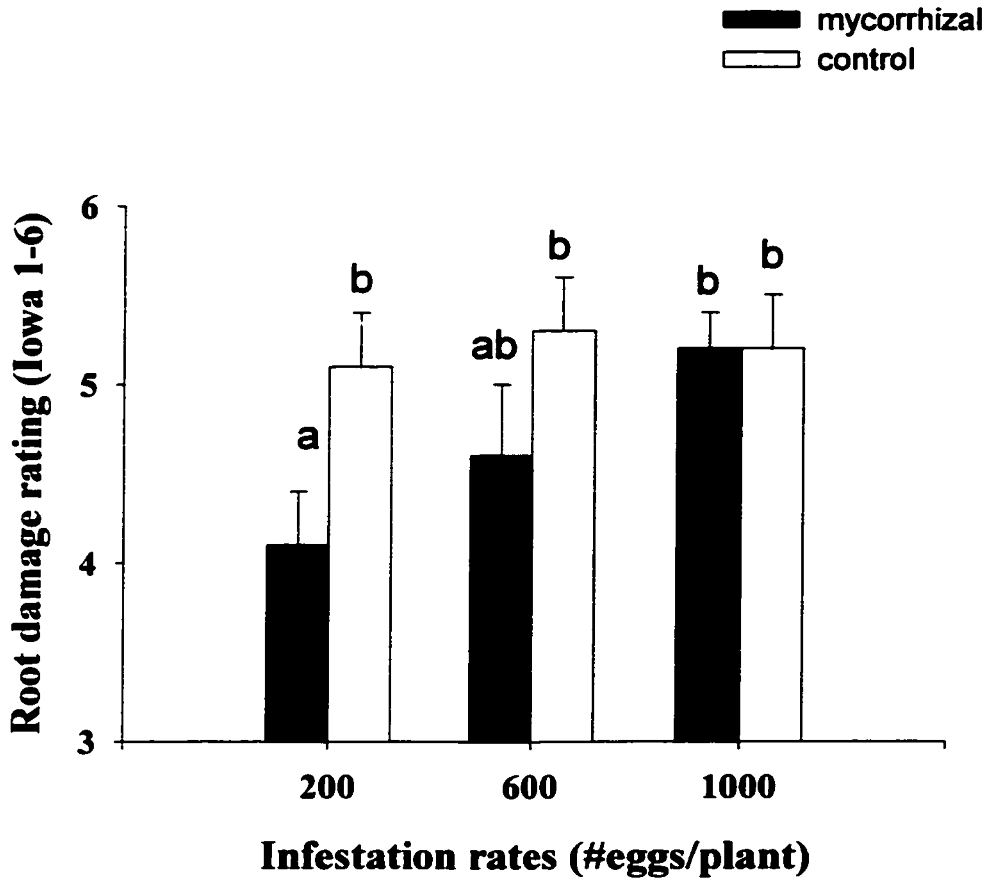

mycorrhizal
control
Stalk circumference (cm)
1,1
0,9
0,7
0,5
a
b
ab
b
b
b
200
600
1000
Infestation rates (#eggs / plant)

Figure 2.6

Effect of mycorrhizal colonization by *G. intrardices* on root damage rating (Iowa 1-6) of maize artificially infested with WCR (1998). The error bars represent the standard error of the mean.


mycorrhizal
control
Root damage rating (Iowa 1-6)
6
5
4
3
a
b
ab
b
b
b
200
600
1000
Infestation rates (#eggs/plant)

especially those grown for grain rather than of sillage, are able to withstand larval root damage rated at 4.0 to 5.0 on the Iowa 1-6 scale (Davis 1994, Sutter et al. 1990). Interestingly, in the 1998 trial, the average root damage ratings of mycorrhizal plants subjected to the low (200 eggs/plant) and medium (600 eggs/plant) infestation rates had in fact been kept below this higher economic damage threshold whereas those of the non-mycorrhizal control plants had not.

There was, however, no observable mycorrhizal effect in the other plant parameters measured in the 1999 trials. This discrepancy in mycorrhizal effects between the two years is explained by a combination of experimental factors: the decreased infestation rates and a reduced egg viability in the second trial.

The infestation rates were reduced in 1999 since those used in 1998 were too high to permit the complete development of WCR larvae into adults. Out of the approximate 19 584 viable eggs applied in both mycorrhizal and control treatments, only 36 adults emerged. This very small emergence rate is most likely the result of extreme intraspecific competition among the WCR larvae within the pots. This explanation is supported by the significant infestation effect on the insect parameters observed (Table 2.2) and the trend shown in Figure 2.7 demonstrating a clear decrease in WCR total mass as infestation rates increase.

Because of the extremely low emergence numbers in the first greenhouse trial, it was impossible to do the appropriate statistical analyses of the emerging adult parameters. Therefore, because of the lower infestation rates and the reduction in viability of the eggs received in 1999, the WCR stress applied to the maize was too mild to observe a mycorrhizal effect on the plant growth parameters.

However, the advantage sought by the reduction in infestation rates was obtained

Table 2.2: Summary of mycorrhizal and infestation rate effects on emerging adult WCR (see appendix ANOVA tables). HCD = head crown diamete; MYC = mycorrhizal treatment; WCR = infestation rates; M*W = mycorrhizal and infestation interaction.

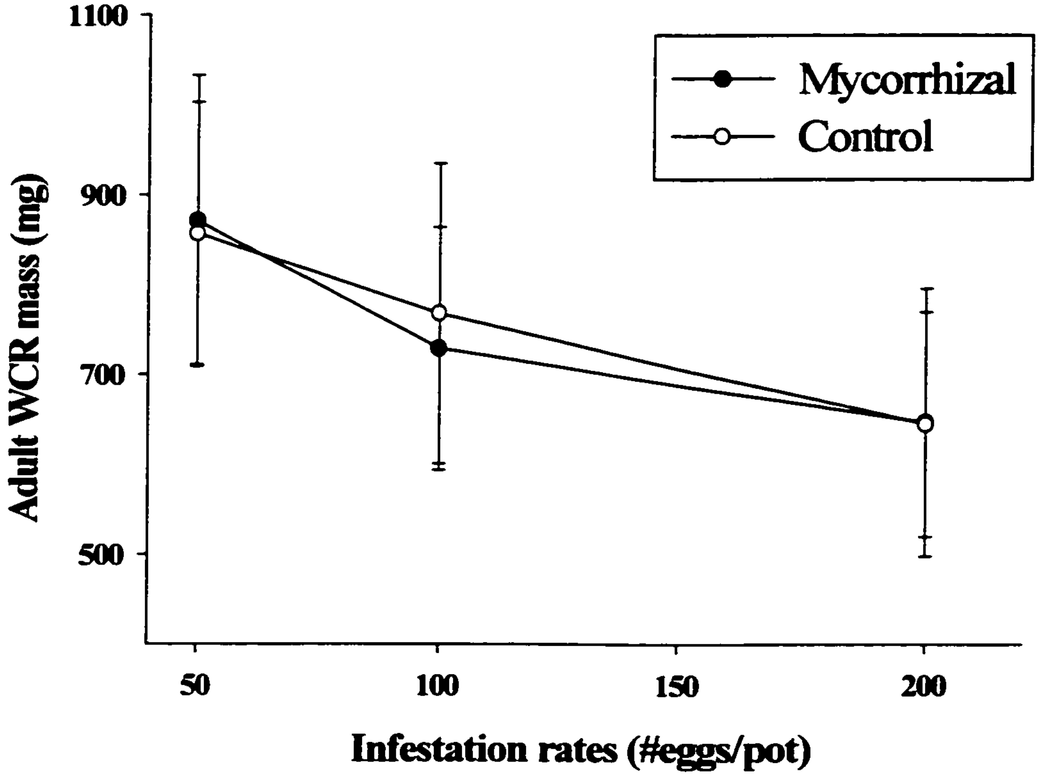
Variables	**p- values (1998)**			**p-values (1999)**		
	MYC	**WCR**	**M*W**	**MYC**	**WCR**	**M*W**
Emerging adult mass	n/a	n/a	n/a	ns	***	ns
Emerging adult HCD	n/a	n/a	n/a	**	***	*

Statistical significance compared to controls: * $p < 0.1$; ** $p < 0.05$; *** $p < 0.01$; ns: $p > 0.1$ n/a: not available

Figure 2.7

Effect of infestation rates on mass of adult WCRs emerging from mycorrhizal and control maize plants.

The error bars represent the standard deviation of the means.


1100
900
700
500
Adult WCR mass (mg)
50
100
150
200
Infestation rates (#eggs/pot)
Mycorrhizal
Control

when a much higher emergence occurred in the 1999 trial. From these adults, a significant mycorrhizal effect on WCR development (figure 2.8) was observed. The head capsule diameters for the insects emerging from mycorrhizal root systems were smaller than those emerging from the control root systems. The greatest difference was observed at the 100 eggs/pot infestation rate.

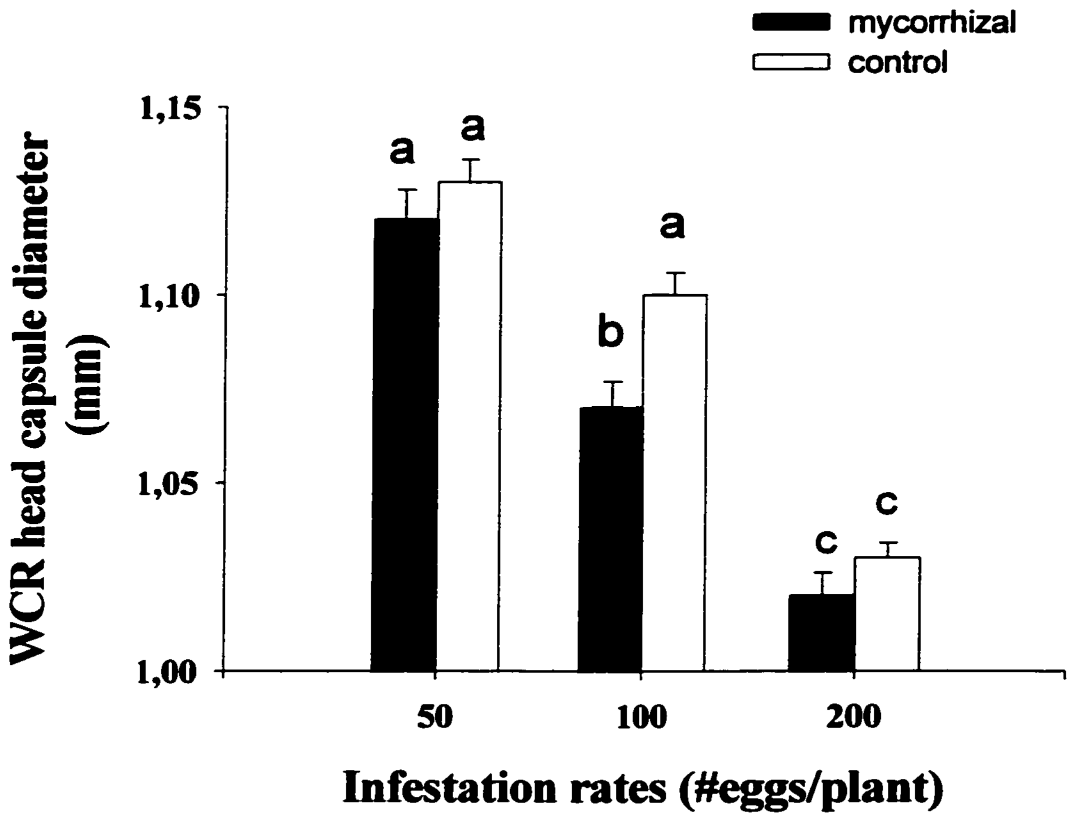
The mechanism by which mycorrhizal colonization increases maize resistance to WCR-induced stress remains to be elucidated. Since larval damage reduces the roots' ability for water uptake, mycorrhizae may be alleviating WCR stress in the same way it does in the case of drought stress where the hyphal network is able to partly compensate for root loss.

A second possibility is that by penetrating the host roots, the fungus is inducing maize defence compounds toxic to the rootworm larvae. Some maize defence compounds such as hydroxamates have been shown to inhibit growth of certain pathogenic fungi (Wilkes t al. 1999). This mechanism could explain the reduction in head crown capsule diameter observed in the WCR adults emerging from mycorrhizal maize. Hydroxamic acids have been shown to negatively affect WCR growth parameters (Xie et al. 1992)

A possible mechanism behind the reduction in larval development is the induction of maize root secondary defence compounds at the time of fungal penetration into the root system. No mycorrhizal effect was observed in the WCR total mass. This result, however, is not surprising since this parameter would be expected to vary more between the time of emergence and the time of capture since this measure does not take feeding by the adult during that time period into account.

Figure 2.8

Effect of mycorrhizal colonization by G. intrardices on head capsule diameter of adult WCR emerging from artificially infested maize plants (1999). The error bars represent the standard error of the mean.


mycorrhizal
control
WCR head capsule diameter (mm)
1,15
1,10
1,05
1,00
a
a
a
b
c
c
50
100
200
Infestation rates (#eggs/plant)

2.3.1. Conclusion

The results obtained from this experiment support both of our original hypotheses in that a mycorrhizal effect beneficial to the maize plants and deleterious to the development of the WCR was observed.

The trends observed in this experiment suggest that the mycorrhizal effect is only observable in the plant parameters when these are grown within a certain range of stress level. If the stress level is above this range, the plants are simply overwhelmed and are unable to benefit from the mycorrhizal symbiosis. This seems to be the case in the high and medium infestation rates from the 1998 trial where, unlike in the low infestation rate, the mycorrhizal effect is insignificant. Moreover, if the stress level is below this range, the plants are unaffected by the larval damage and therefore do not profit from the compensatory effects of the mycorrhizae. This would explain the insignificant differences between the mycorrhizal and control maize from the 1999 trial.

A single adult WCR female has a lifetime fecundity of more than 1000 eggs (Branson & Johnson 1973). A reduction of the WCR reproductive potential could be a powerful element in the management of this pest. It would therefore be of great value to understand the effect of mycorrhizae on the insect's post-emergence survival and reproductive potential. Future experiments should be done to test for a possible reduction of female fecundity and fertility.

CHAPTER III

Analyses of 2,4-Dihydroxy-7-methoxy-2H-1,4benzoxazin-3-one (DIMBOA) and 6-methoxybenzoxaxolinone (MBOA) in Mycorrhizal Maize Roots

3.1. Introduction

Our previous results have shown that mycorrhizal colonization reduced maize susceptibility to stress caused by the feeding of WCR larvae. A negative mycorrhizal impact on larval development was also observed. Various mechanisms explaining the mycorrhizal action have been proposed (St-Arnaud et al. 1995). Among these is an improvement of the water and nutrient status of the mycorrhizal host plants compared to the non-mycorrhizal control plants. In this case, the extended mycorrhizal hyphal network is able to compensate for the damaged root system of the maize plants by greatly increasing its absorptive surface. Although this mechanism is able to explain the beneficial effects of VAM colonization on infested maize plants it is less likely that the detrimental effect observed in the WCR adults is a direct result of improved plant nutrition and water status.

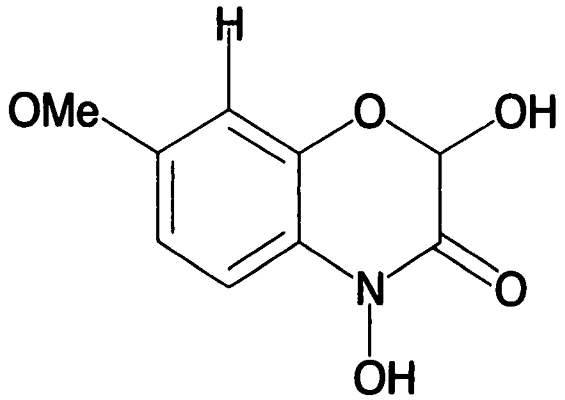
Therefore, a second mechanism is proposed by which there is an induction of plant secondary defence compounds caused by early mycorrhizal colonization of roots. This type of mechanism could explain the reduction in WCR head capsule diameters observed in the mycorrhizal treatments reported in the previous experiment (Chap. II). The concept of a systemic acquired resistance or SAR (Ross 1961) in plants has been demonstrated repeatedly. Induced modifications in the accumulation patterns of root secondary compounds such as phenolics by early mycorrhizal fungus colonization have previously been observed (Peip et al 1997). Such accumulations are believed to result from the triggering of plant defence mechanisms by the initial stages of fungal colonization of the roots.

The goal of the current experiment was to test for a possible induction of secondary defence compounds by mycorrhizal colonization in maize roots. Hydroxamic acids, specifically 1,4-benzoxazin-3-ones, are known resistance factors commonly found in several species of the Poaceae family, including *Zea mays* (Niemeyer 1988). The main hydroxamic acid found in maize roots is 2,4-dihydroxy-7-mehoxy-(2H)-1,4-benzoxazin-3(4H)-one, DIMBOA (see figure 3.1 for structure). The experiments by Klun et al. (1967) were the first to demonstrate the protective role of DIMBOA against important pests such as the European Corn Borer.

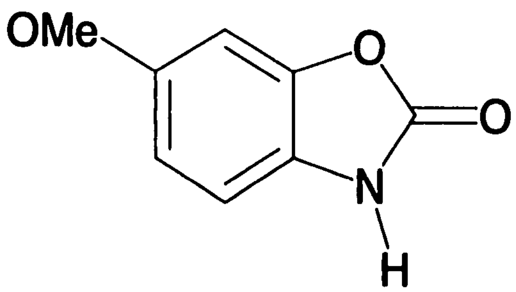
DIMBOA degrades into formic acid and its major degradation product, 6-methoxybenzoxazolinone (MBOA) (see figure 3.1 for structure). MBOA is only found in fresh maize tissue only as the result of DIMBOA degradation (Hofman and Hofmanova, 1971). However, the conversion of DIMBOA to MBOA is not stoichimetrical. Woodward et al. (1978) demonstrated that the quantity of MBOA yielding from original DIMBOA concentrations was highly dependent on pH and temperature. The conversion rates of DIMBOA to MBOA decrease with decreasing pH (Woodward et al. 1978, Bravo & Niemeyer 1986)

Figure 3.1:

Chemical structure of 2,4-hydrox-7-mehoxy-1,4-benzoxazin-3-(4H)-one (DIMBOA) and 6-methoxybenzoxazolinone (MBOA)



DIMBOA



MBOA

Hydroxamic acid levels have been shown to increase as an induced response (IR) in plants subjected to abiotic (Richardson and Bacon 1993) and biotic stresses (Gianoli & Niemeyer 1997, Niemeyer et al. 1989). Moreover, Xie et al. (1990) have demonstrated a detrimental effect of DIMBOA, the principal hydroxamic acid present in maize roots, on WCR larvae. The benzoxazolinone MBOA, the main degradation product of DIMBOA, also showed toxic effects towards the larvae in their experiment. The effects of the hydroxamic acids resulted in a reduction of the average head crown capsule diameters.

The underlying hypothesis in this experiment is that mycorrhizal colonization reduces maize susceptibility to WCR larvae feeding stress by modifying accumulation of Hydroxamic acids (Hx) in host plant roots. From this hypothesis, it is predicted that the early colonization of maize by the VAM fungus, *Glomus intraradices* will have an inducive effect on the production of two major hydroxamic acids, 2,4-dihydrox-7-methoxy-1,4-benzoxazin-3-(4H)-one (DIMBOA) and 6-methoxybenzoxazolinone (MBOA) in the roots. It is also known that maize root Hx levels vary greatly in the first 4 weeks after germination. Xie et al. (1992) determined that total Hx levels were at their highest in 2 week old maize roots whereas DIMBOA levels peaked at 4 weeks.

3.2. Method and material

3.2.1. Plant material and growth conditions

The maize hybrid Pioneer 3921 was grown for this experiment under greenhouse conditions in March of 2000. The plants were grown in a substrate consisting of equal parts peat moss, top soil and perlite. These three components were mixed and sterilised in an autoclave prior to planting. The 6L pots were filled with 4 litres of the growth substrate and then 250ml of commercial soil- based inoculum purchased from Premiertech® was added. The inoculum contained propagules from the mycorrhizal fungus *Glomus intrardices*. The same soil-based inoculum without fungal propagules was added in the same way to the control pots for the non-mycorrhizal treatment. Two litres of growth substrate were then added to fill each pot. Three maize seeds were planted in each of the 6L pots. The light regime was 14:10 (light:dark) under natural light. No artificial lighting was used. The pots were arranged in a complete randomized block.

3.2.2. Mycorrhizal colonization evaluation

Three plants from both mycorrhizal and control treatments were left to grow for 10 weeks in order to evaluate mycorrhizal colonization. Root staining and mounting was done according to the method previously described (Chapter 2). A total of 70 root segments were used for each of the 6 plants.

3.2.3. Extraction for HPLC analyses

From 5 plants/treatment, 3 x 1g of fresh root was weighed, giving a total of 15 replicates per treatment. The fresh material was ground using a mortar and pestle in 3X5 ml of distilled water. The slurry was left to stand at room temperature for 12 hours to allow for enzymatic hydrolysis of the DIMBOA glucoside. The samples were sonicated for 3 minutes in a Branson 2000 Ultrasonic Cleaner and then filtered through 3 layers of bleached cheese cloth. The residue was discarded. The filtrate (~pH 5.8) was brought to pH 2 with 2N HCl then heated to 65C for 1 minute and cooled in ice water for 10 minutes in order to precipitate proteins. The samples were filtered through Whatman 42 filter paper and extracted in a separatory funnel with 10 ml Ethyl Acetate (2X). The aqueous fraction was discarded whereas the organic phases were pooled. The pooled organic phase was evaporated under vacuum at 40°C with a Yamamato RE 500 rotary evaporator and dried completely under a stream of nitrogen, then resuspended in 1ml methanol (2X; pooled) and stored under nitrogen at -20C until HPLC analysis. The extraction procedure is summarized in figure 3.2.

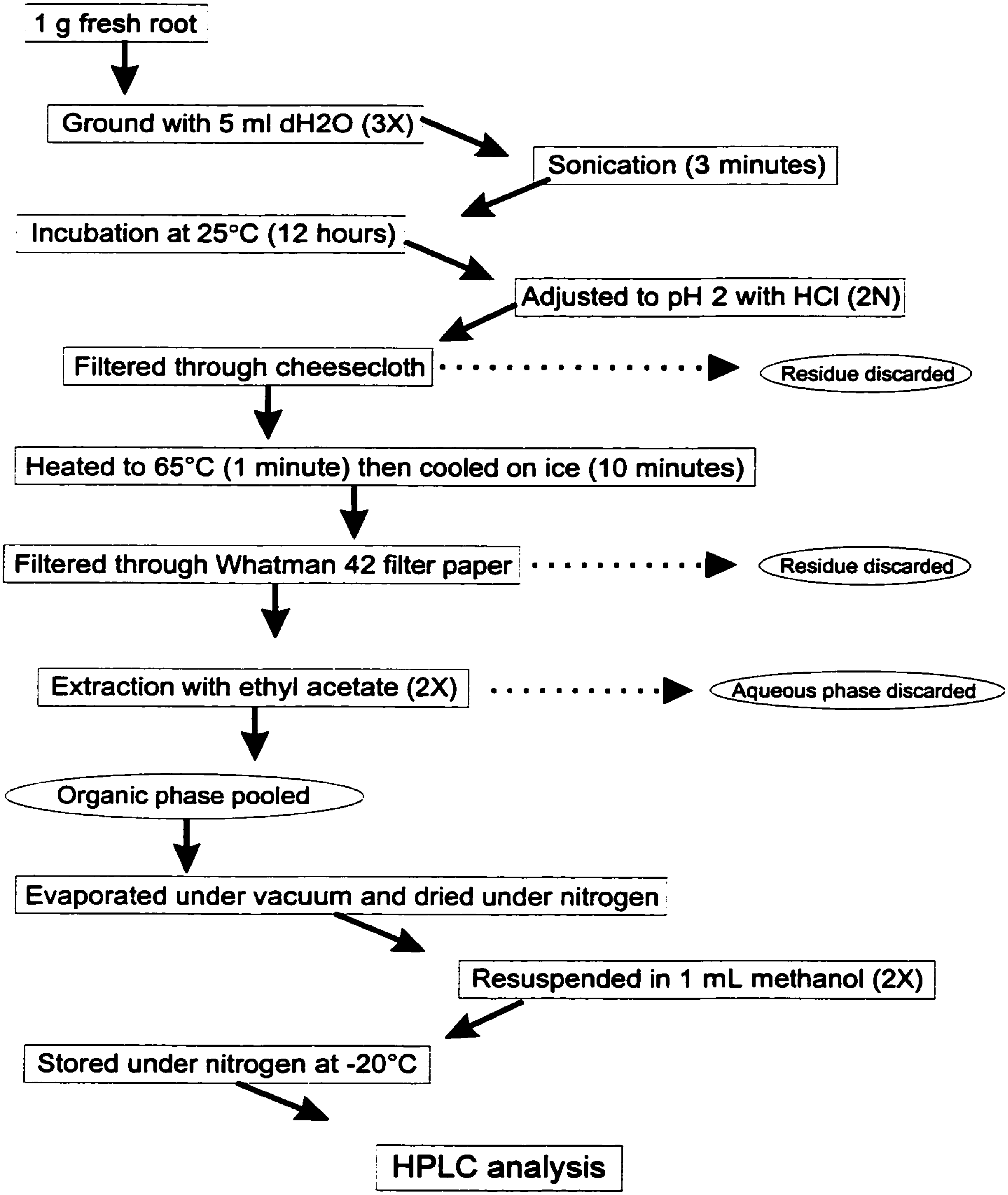
3.2.4. HPLC analyses and instruments

The samples were removed from the freezer and dried using a Savant Automatic Environmental Speedvac for 25 minutes under full vacuum with the Radiant Cover disabled at a medium drying rate (43C). These were then resuspended in 2 ml of HPLC grade MeOH. Samples were filtered using a 3ml Luer-Lok Latex Free Syringe and a 0.45 µm syringe filter.

HPLC analysis was done using an Agilent 1100 Series Quaternary Pump equipped with an Agilent 1100 Series Diode Array Detector (DAD) and an Agilent 1100 Series Autosampler. The column used was an Alltech Hypersil ODS C18 3µ with a length fo 100mm and an internal diameter of 4.6mm. The injection volume for each sample was 5µl and with a flow rate of 1ml/min. The mobile phase consisted of HPLC grade MeOH (Solvent A) and 25 mM NaH_2PO_4 buffer solution at pH 2.3 (Solvent B). The gradient was as follows: initial conditions were 25% Sol.A and 75% Sol.B.; Solvent A was increased

Figure 3.2

Flow chart of hydroxamic acid extraction procedure for roots of greenhouse grown maize


1 g fresh root
Ground with 5 ml dH2O (3X)
Sonication (3 minutes)
Incubation at 25°C (12 hours)
Adjusted to pH 2 with HCl (2N)
Filtered through cheesecloth
Residue discarded
Heated to 65°C (1 minute) then cooled on ice (10 minutes)
Filtered through Whatman 42 filter paper
Residue discarded
Extraction with ethyl acetate (2X)
Aqueous phase discarded
Organic phase pooled
Evaporated under vacuum and dried under nitrogen
Resuspended in 1 mL methanol (2X)
Stored under nitrogen at -20°C
HPLC analysis

linearly over 10 minutes to 45% and then to 80% over 2 minutes. This gradient was maintained for 3 minutes, then initial conditions were reestablished over 2 minutes and maintained for 8 minutes.

3.2.5. DIMBOA and MBOA quantification

Retention times and response factors for DIMBOA and MBOA were determined from standards synthesized by Atkinson (1989). The UV spectra were also compared to further support compound identification. UV spectra for DIMBOA and MBOA are presented in figure 3.3.A. The DAD was set to record signals at 210, 263 and 295 nm.

In order to obtain a more accurate evaluation of total DIMBOA concentrations in roots before extraction, DIMBOA equivalents were also determined. Since DIMBOA conversion to MBOA is non-stoichimetric the results obtained by Woodward et al. (1978) were used and a linear relationship between temperature and MBOA yield percentage was assumed to obtain a conversion rate. The estimated rate of conversion of DIMBOA to MBOA used in the DIMBOA equivalents calculations was estimated at 62.4% for our extraction conditions (25C, pH 5.5).

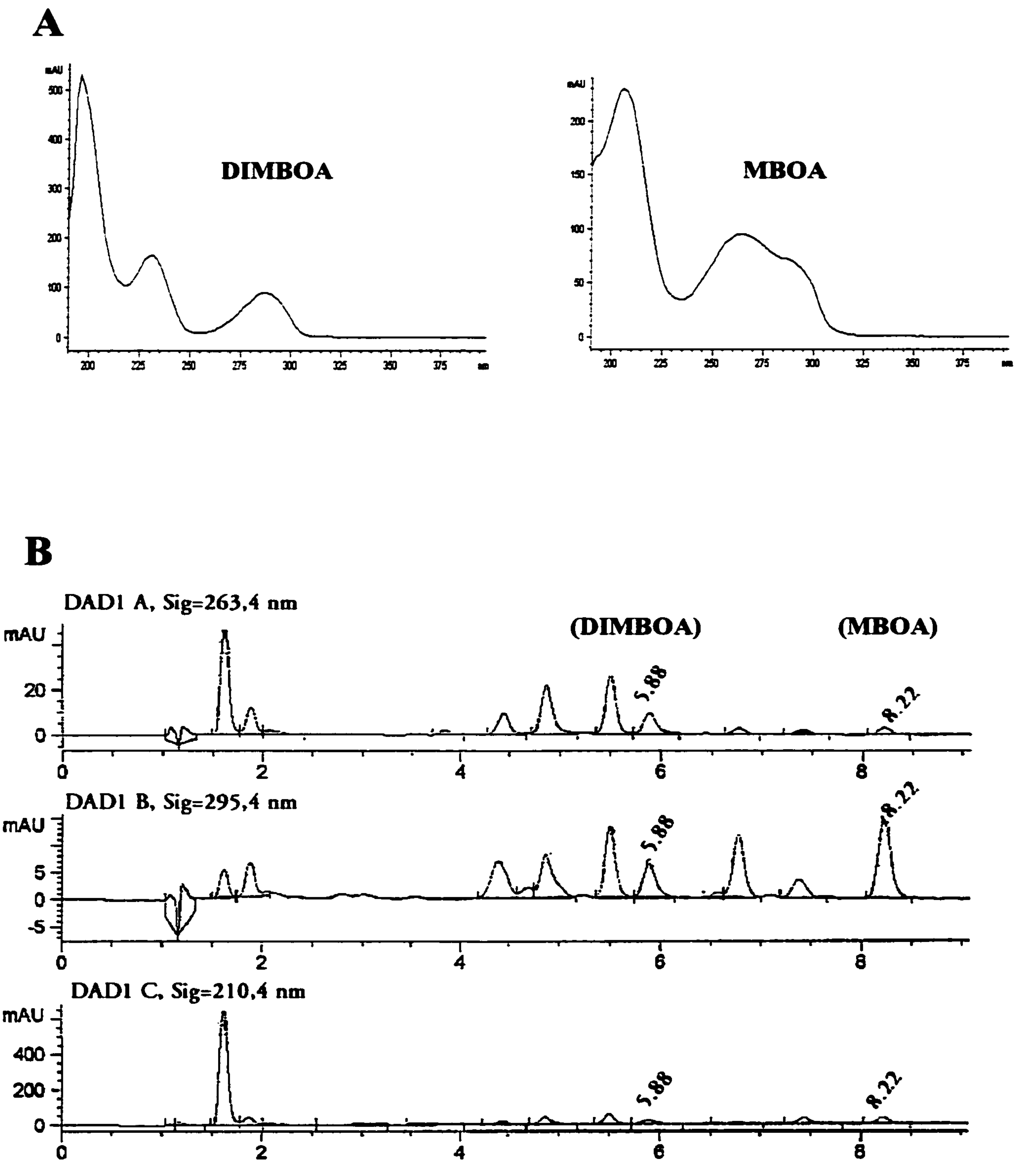
3.2.6. Statistical analysis

The data were tested for normality and homogeneity of variance. The data, having met the assumptions, were then analysed using a two-way parametric ANOVA with mycorrhizal colonization and plant age as the two main effects. The dependent variables were either DIMBOA, MOBA or DIMBOA equivalents..

Figure 3.3:

A-UV spectra for 2,4-hydroxy-7-methoxy-1,4-benzoxazin-3-(4H)-one (DIMBOA) and 6-methoxybenzoxazolinone (MBOA);

B-Example of HPLC chromatogram for DIMBOA (retention time: 5.88 min.) and MBOA (retention time: 8.22 min.) extract of maize roots seperated by the methods described in the text and analysed at 3 different wavelengths (263.4 nm, 295.4 nm and 210.4 nm)


A
mAU
500
400
300
200
100
0
200
225
250
275
300
325
350
375
nm
DIMBOA
mAU
200
150
100
50
0
200
225
250
275
300
325
350
375
nm
MBOA
B
DAD1 A, Sig=263,4 nm
(DIMBOA)
(MBOA)
mAU
20
0
5.88
8.22
0
2
4
6
8
DAD1 B, Sig=295,4 nm
mAU
5
0
-5
5.88
8.22
0
2
4
6
8
DAD1 C, Sig=210,4 nm
mAU
400
200
0
5.88
8.22
0
2
4
6
8

3.3. Results and Discussion

At 10 weeks, overall mycorrhizal colonization percentage in the inoculated plants was evaluated at 29%. The maize plants grown under non-mycorrhizal control conditions showed no mycorrhizal formation.

Figure 3.3.B shows an example of a typical chromatogram obtained from a DIMBOA and MBOA extract of maize roots. The mycorrhizal roots harvested at 2 weeks after germination showed higher levels of MBOA than did the control plants. MBOA concentrations in the roots of the 4 week old plants, although slightly higher in the mycorrhizal plants, showed no significant differences between the two treatments (Table 3.1 & figure 3.4). No statistically significant differences were observed among any of the DIMBOA concentrations (Table 3.2 & figure 3.5).

Since the conversion of DIMBOA to MBOA is known to be considerably less than 100% and varies with pH and temperature (Woodward et al. 1978), the DIMBOA equivalents measured in this experiment give a better estimation of total DIMBOA levels found in the roots before the extraction procedures. It was found that with the DIMBOA equivalents, the age of the plant had a significant effect on concentrations found in the fresh maize roots. The roots analysed at 4 weeks of age showed a mean increase in DIMBOA equivalents levels of almost 16% when compared to roots analysed at 2 weeks of age. This result is similar to that reported by Xie et al. (1992) who observed peak DIMBOA equivalents concentration at 4 weeks with statistically significant differences between levels observed at 2 and 4 weeks of age (Table 3.3 & figure 3.6).

When comparing concentrations of DIMBOA equivalents between mycorrhizal and control treatments at the two different ages, there is a persistent trend where higher

Table 3.1 Effects of mycorrhizal colonization and plant age on extracted MBOA accumulation in maize roots at 2 and 4 weeks

	Analysis of variance			
	df	Mean Square	F ratio	P
Mycorrhizal	1	31.813	3.416	0.070
Age	1	187.034	20.083	0.000
M * A	1	4.785	0.514	0.476
Error	59	9.313		

Figure 3.4:

Effect of mycorrhizal colonization on extracted MBOA concentrations of maize roots 2 weeks and 4 weeks of age. Error bars represent the standard error of the mean.

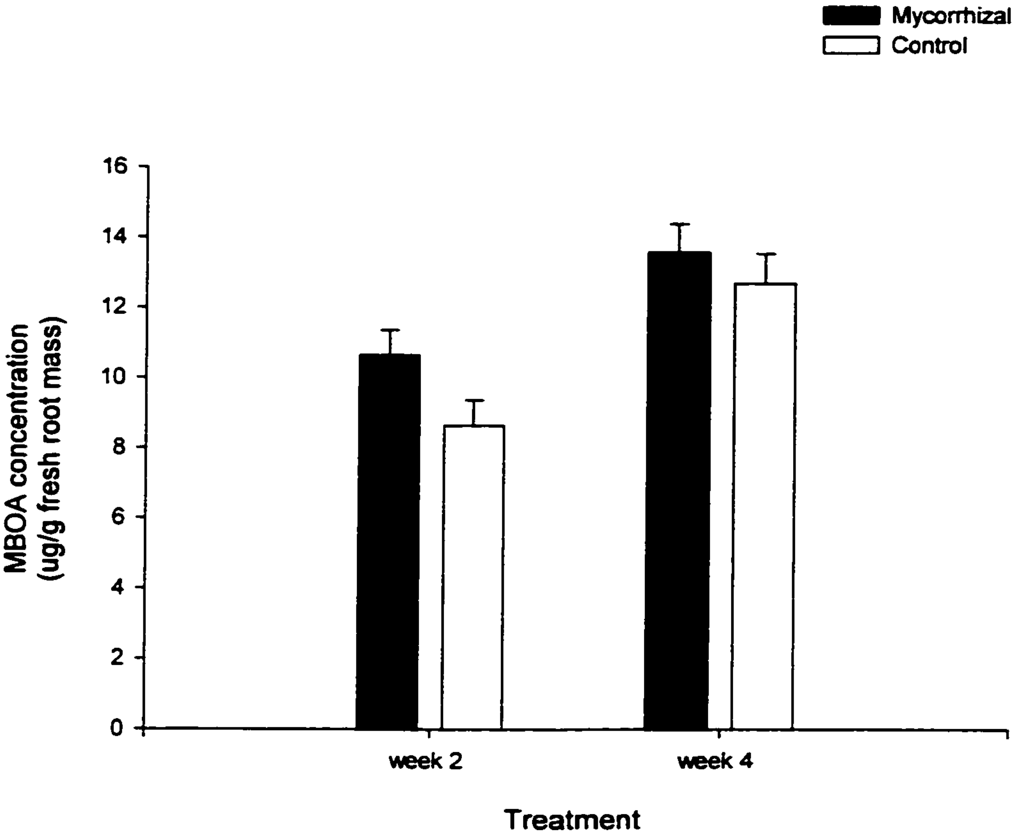
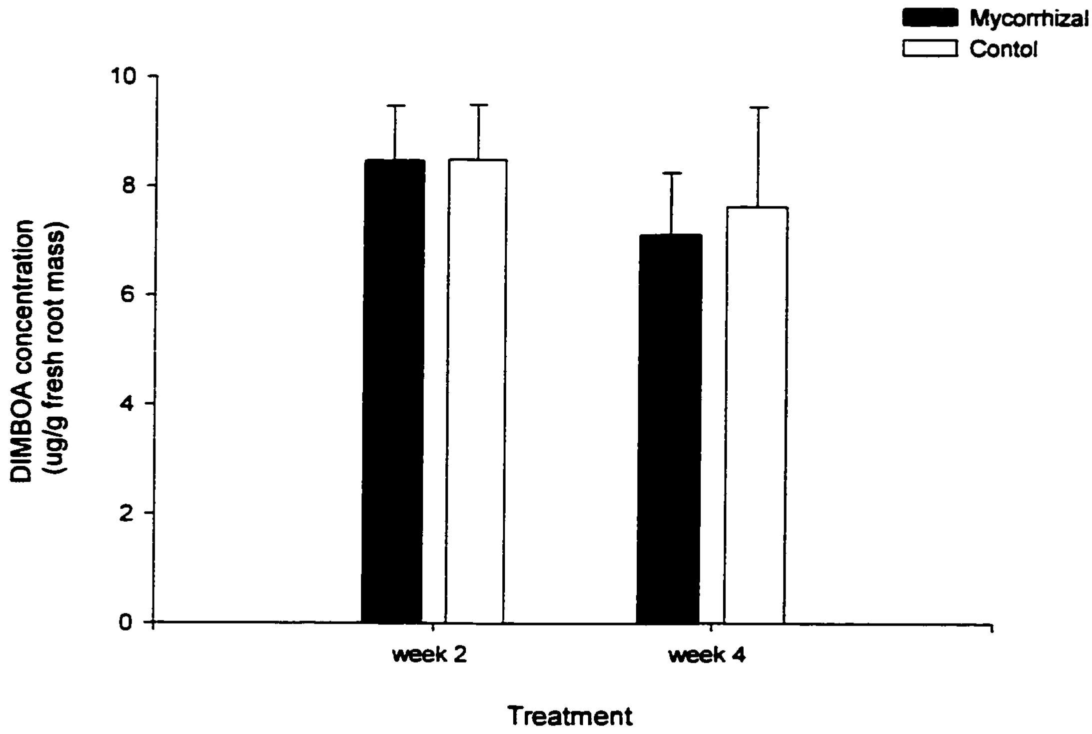

Mycorrhizal
Control
MBOA concentration (ug/g fresh root mass)
0
2
4
6
8
10
12
14
16
week 2
week 4
Treatment

Table 3.2 Effects of mycorrhizal colonization and plant age on extracted DIMBOA accumulation in maize roots at 2 and 4 weeks

	Analysis of variance			
	df	Mean Square	F ratio	P
Mycorrhizal	1	1.128	0.062	0.804
Age	1	18.886	1.040	0.312
M * A	1	0.952	0.052	0.820
Error	59	18.163		

Figure 3.5:

Effect of mycorrhizal colonization on extracted DIMBOA concentrations of maize roots 2 weeks and 4 weeks of age. Error bars represent the standard error of the mean.



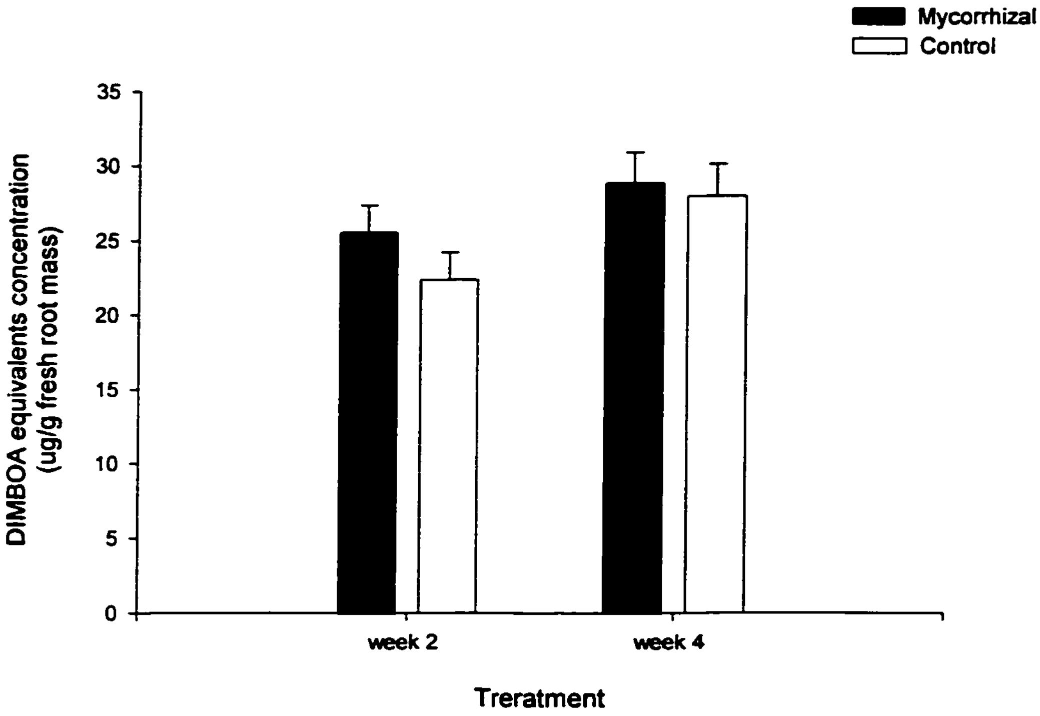
■ Mycorrhizal
□ Contol
10
8
6
4
2
0
DIMBOA concentration (ug/g fresh root mass)
week 2
week 4
Treatment

Table 3.3 Effects of mycorrhizal colonization and plant age on DIMBOA equivalents accumulation in maize roots at 2 and 4 weeks

	Analysis of variance			
	df	Mean Square	F ratio	P
Mycorrhizal	1	63.628	1.048	0.310
Age	1	308.739	5.087	0.028
M * A	1	20.084	0.331	0.567
Error	59	60.686		

Figure 3.6:

Effect of mycorrhizal colonization on DIMBOA equivalent concentrations of maize roots 2 weeks and 4 weeks of age. Error bars represent the standard error of the mean.


Mycorrhizal
Control
35
30
25
20
15
10
5
0
DIMBOA equivalents concentration (ug/g fresh root mass)
week 2
week 4
Treratment

concentrations are observed in the mycorrhizal maize roots than in the non-mycorrhizal maize roots. Statistical analyses, however, indicate that the differences observed are not significant at the accepted critical p-value.

It is believed that stress intensity has an influence on the magnitude of the IR in plants (Karban 1991) The increased levels of Hx as an IR in plants has specifically been shown to be affected by the intensity level of the imposed stress. Gianoli & Niemeyer (1997) demonstrated a herbivory intensity threshold under which Hx levels in stressed plants did not differ significantly from the Hx levels in the control plants. These previous results raise the distinct possibility that maize root response to mycorhizae is also affected by the intensity of the perceived attack, early fungal colonization, and that higher levels of early mycorrhizal colonization could indeed lead to statistically significant effects on DIMBOA accumulation in roots.

Evidence has shown that early mycorrhizal colonization of roots can trigger defence processes similar to those observed in pathogenic infection (Perrin 1990). Peipp et al. (1997) recently observed fungus-induced accumulations of hydroxycinnamic acid amides in *Hordeum vulgare* roots during the early stages of mycorrhizal establishment.

3.3.1. Conclusion

Although differences obtained in this experiment were not statistically significant, the overall trends observed were similar to what was predicted by our initial hypothesis that early mycorrhizal colonization would lead to the induction of secondary defence compounds such as DIMBOA, an important compound for macro-invertebrate pest resistance in maize roots.

However, since the differences observed in DIMBOA equivalents were not significantly different, it is doubtful that the hypothetical mechanism proposed in this experiment accounts for the reduction in emerging WCR head capsule diameter.

Future work in this field should focus on correlating the effects of mycorrhizal colonization levels and the magnitude IR observed. It would also be important to test the effects of mycorrhizal colonization on other inducible secondary defence compounds. This information could help elucidate clearly the mechanism(s) by which mycorrhizal colonization reduces maize susceptibility to WCR larval feeding damage.

CHAPTER IV

Summary & Conclusion

This study has focussed on the impact of mycorrhizae as a tool for pest control. Even though experiments were carried out under greenhouse controlled conditions, it is possible to foresee what would occur under field conditions.

Based on the results reported, the following conclusions have been drawn:

1- In our greenhouse trials, a beneficial effect of mycorrhizal colonization on maize plant parameters under WCR larval feeding stress was found (Chapter II).

2- The mycorrhizal effect observed in the greenhouse trial experiments appears to have a stress threshold; if exceeded, beneficial effects of the mutualistic symbiosis is lost (Chapter II).

3- A deleterious effect of the mycorrhizae on the development of the emerging adults has been demonstrated, the most prominent feature being a reduction in the head capsule diameters of the insects emerging from mycorrhizal root systems (Chapter II).

4- An overall trend was observed in the hydroxamic acid levels. This suggests a possible mechanism behind the observed effects of mycorrhizae on the maize-rootworm interaction (Chapter III).

The significance of our findings is discussed below.

The beneficial effects to the maize plants derived from AM association observed in the first greenhouse trial were apparent only in the lower infestation rates (200 & 600 eggs/plant). This raises questions about the suitability of mycorrhizae as effective biologically derived control elements in fields subjected to heavy WCR infestations. Conversely, no mycorrhizal effect was observed in the second greenhouse trial where infestation rates were reduced in order to increase adult emergence rates. These observations lead us to believe that a maize plant's ability to benefit from a reduction in susceptibility to WCR attack derived from mycorrhizal mutualism is limited to a window of stress intensity.

One of the more significant and potentially beneficial effects supporting the adoption of mycorrhizal-conducive practices for maize cultivation in regards to WCR management is the reduction of root damage ratings to below accepted economic damage thresholds (Davis 1994, Sutter et al. 1990). Results obtained from our greenhouse trials clearly demonstrated such a mediative mycorrhizal impact on maize plants coping with WCR larval feeding stress. Gray et al. (1992) were able to show through participatory on-farm experiments in Illinois that labelled application rates for soil insecticides registered for *Diabrotica* spp. were excessive and could be considerably reduced without incurrence of economic damage to maize production. In light of the evidence related to root damage presented in this thesis, it seems quite possible that mycorrhizal mutualism in maize crops could, if not further reduce the use of soil insecticides, certainly increase their efficiency at presently recommended rates. The potential for cost reduction to maize producers in such a case is clear.

Xie et al (1990 & 1991) observed a reduction in larval head-capsule diameter in maize lines with roots having high DIMBOA concentrations. These results are similar to our current findings. DIMBOA has been shown to decrease the digestibility of diet to a variety of insects, while MBOA appears to reduce the conversion of digested food into net biomass (Houseman et al. 1992). These two modes of action of Hx may be responsible for the smaller emerging adults in the mycorrhizal treatments. MBOA has been recognized as a maize semiochemical for WCR larval location of host roots (Hibbard & Bjostad 1990, Bjostad & Hibbard 1992). Abou-Fakhr et al. (1994) have reported the absence of toxic effects of MBOA to WCR larvae. Toxicity in this study however was evaluated through larval mortality and behavioural changes only. Larval development and size was not reported. Taking into account the likely mode of action of MBOA previously mentioned, it is reasonable to believe that deleterious effects of this semiochemical might only be observed through size reductions in subsequent life stages.

The induction of other secondary compounds as well as the Hx DIMBOA and its derivatives in response to mycorrhizal colonization could explain the reduction in larval development and may in turn lead to the reduction of WCR adult fitness. This idea is supported by the trends obtained from the Hx profiles of mycorrhizal and non-mycorrhizal maize roots reported in chapter 3. Various other plant responses influenced by fungal colonization could potentially impact WCR development through mechanisms of cross protection. Among these is the production of phytoalexin, modifications to root cell walls and the triggering of systemic resistance mechanisms by wound-induced signals such as jasmonates and salicylic acid (Hammerschmidt & Schultz, 1996). Enhanced levels of root pathogenesis-related proteins such as endochitinases have been observed during early colonization stages of *G. intraradices*.(Lambais & Mehdy, 1993, Spanu et al. 1989, Volpin et al. 1994).

4.1. The role of arbuscular mycorrhizae in plant resistance to biotic stress

The findings of this study have further demonstrated the role that VA mycorrhizae are able to play in reducing the susceptibility of plants to biotic stress. Whereas most of the literature on such mycorrhizal effect has revolved principally around the plant-fungal interaction in relation to microscopic pathogens (i.e. fungus, virus, bacteria and nematodes), this study has shown a mycorrhizal importance in the mediation of stress caused by a macroscopic root-born pest, the Western Corn Rootworm.

It is important to note that the negative impact of injury caused by WCR larvae to maize roots is not solely limited to the direct effects of lost and damaged root mass. In many cases, susceptibility to root and stalk rot fungi in plants hosting WCR larvae is dramatically increased (Palmer and Kommedahl 1969). However, several studies have already shown a reduction in pathogenic fungal effects due to VA associations (Dehne 1982, Caron 1992, St-Arnaud et al. 1995). From this, another potential mode of endomycorrhizal protection of maize seems apparent.

In addition to direct beneficial effects on crop plants, Bethlenfalvay & Linderman (1990) have suggested that mycorrhizae may also be of great importance to sustainable agriculture due to their ability to regenerate cultivated soils that have been consistently degraded by modern agricultural practices.

4.2. The use of mycorrhizae for agricultural applications

The current study underlines potential benefits of adopting agricultural strategies and practices conducive to the establishment and maintenance of functional mycorrhizal symbioses between crop plant roots and mycorrhizal fungus. Arbuscular mycorrhizal associations are naturally occurring in the large majority of terrestrial plants and AM propagules are ubiquitously present in almost all types of soils. It is for these reasons that in maintaining the principles of IPM, efforts should be placed on the conservation of the endogenous mycorrhizal populations in agricultural soils as opposed to the introduction of exogenous sources of mycorrhizae, thereby reducing inputs and in turn, costs to producers. However, many factors must be taken into account when weighing the merits of promoting indigenous fungal population in opposition to inoculation. Among these factors is the composition of the natural mycorrhizal population, the mycorrhizal dependence and responsiveness of the crop plant in question, effects of existing soil management practices and of course, soil composition (Smith & Read 1997).

Despite the ever increasing evidence supporting the exploitation of mycorrhizal benefits in horticultural and agricultural production, there are few examples of widespread commercial uses of AM (Smith & Read 1997). This in large part is due to the difficulties associated with obtaining and/or producing mycorrhizal inoculum as well as its application to cultivated soils. Mycorrhizae become beneficial, from an economic stand point, when profits due to mycorrhizae-related yield increases surpass the costs of application and management of the mycoflora. Therefore, the ideal scenario for maximum mycorrhizal benefits is that of high value crops requiring minimal changes to current cultivation practices in order to ameliorate their mycorrhizal associations.

The importance of arbuscular mycorrhizae as factors regulating the vitality of agroecosystems is often overlooked and/or underestimated. It has been suggested that this is in large part due to the absence of a convincing case by mycorrhizologists in support of the crucial role of arbuscular mycorrhizae (Sylvia & Williams 1991).

There is an inherent complexity associated with the study of mycorrhizal effects on host plants. Keeping in mind the tri-component nature of mycorrhizae (i.e. plant, fungus and soil) and the various elements affecting each of them (figure 4.1), the possible interactions and variations between the factors regulating the symbiosis appear to be almost overwhelmingly numerous. This, in many cases, is the cause of the difficulty in obtaining clear and convincing results that support the importance of AM mycoflora in agrosystems. In addition, differences in timing and location of the fungal association with the host plant may also impact on the protective mycorrhizal effects observed (ex: Bååth et Hayman 1983, Kaye et al. 1984, Krishna et Bagyaraj 1983).

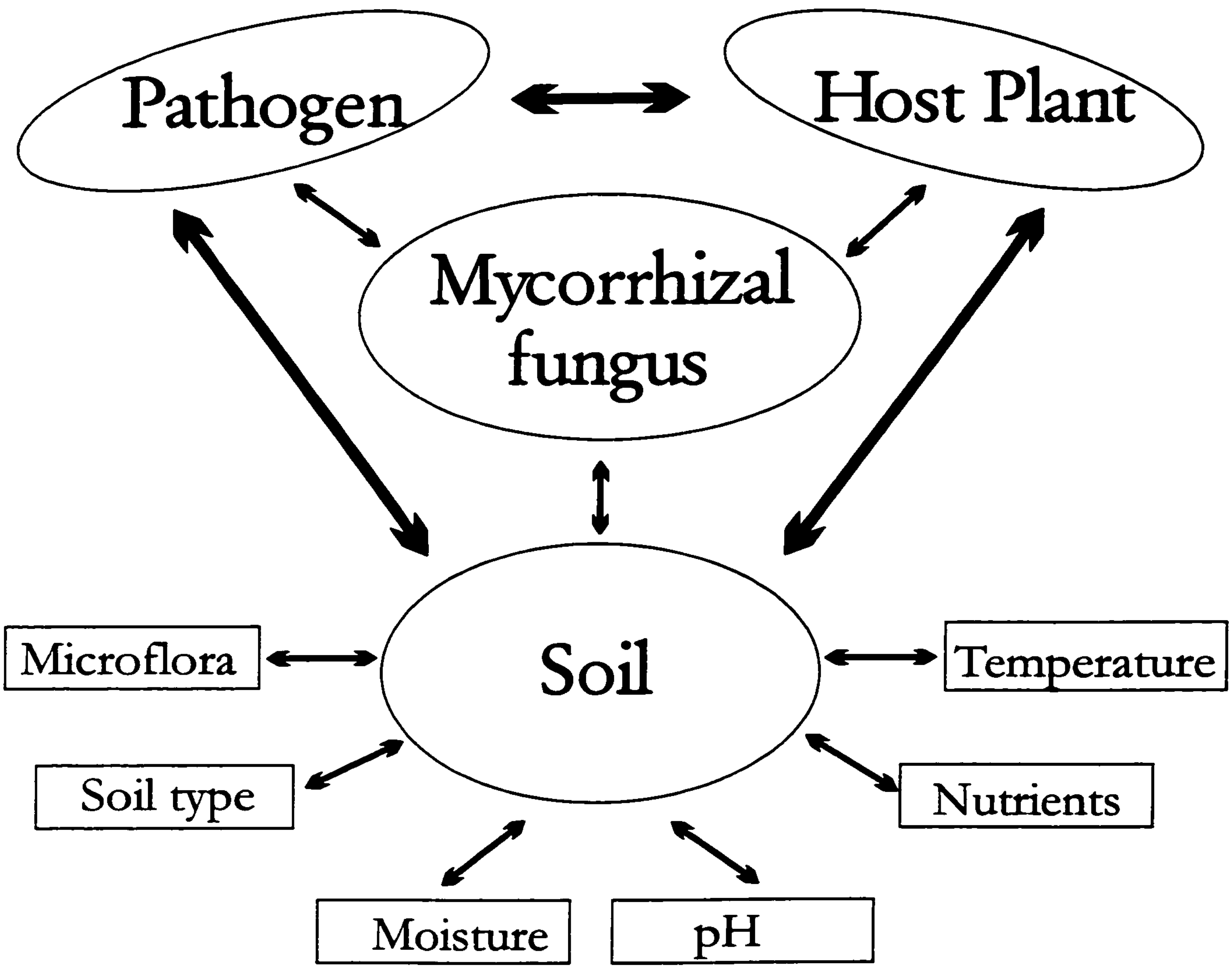
Considerable evidence supports the idea that different fungal species implicated in mycorrhizal associations may have varying effects on various plant species (Abdul and Janardhanan 1997, Dalpé et al. 1996, Silveira and Lima 1996, Ruiz-Lozano et al. 1995) including maize (Boucher et al. 1999, Clark and Zeto 1996). Such variations may also be observed in the protective mycorrhizal effects against WCR and may therefore be maximized, or at least increased with fungal species other than *Glomus intraradices*.

Work on the development of maize hybrids resistant to WCR is presently being done by various agricultural companies using traditional breeding practices as well as direct genetic manipulation through biotechnological techniques. The commercial introduction of a genetically engineered variety of WCR-resistant maize had been expected for the 2001 growing season. Resistance in this variety is conferred through the introduction of a gene from the soil born bacterium *Bacillus thuringiensis* var. *tenebrionis* that encodes a protein

Figure 4.1:

Possible interactions affecting mycorrhizal effects

(Adapted from Caron 1992)


Pathogen
Host Plant
Mycorrhizal fungus
Soil
Microflora
Temperature
Soil type
Nutrients
Moisture
pH

toxic to WCR larvae (Ferber 2000). Despite the tremendous potential benefits of genetically modified maize varieties, many concerns regarding their widespread use are being brought forward by various parties. These concerns include deleterious effects on non-target organisms, hastened development of WCR resistance and popular disapproval.

The ability of WCR to develop resistance against commonly used control practices is high and, to the dismay of maize producers, has repeatedly made itself apparent (see table 4.1 for examples). One of the more recent, and probably most troubling examples of WCR resistance is the emergence of a new strain able to tolerate crop rotation by ovipositing in soybean fields (Sutter 1999). The importance of developing new WCR control strategies and the improvement of the present ones cannot be overstated. The use of mycorrhizal mutualism holds the potential of being a valuable addition to current WCR management strategies since they are able to meet two important criteria of pest control; those being of low cost and environmentally sound.

4.3. Future work

(1). The conclusions drawn from this study are entirely based on controlled greenhouse trials. It goes without mention that for there to be a practical application of mycorrhizal inoculation to reduce maize susceptibility to WCR damage, the effects observed in this work should now be observable in a realistic agricultural environment. Field studies must therefore be undertaken in order to verify and support our greenhouse findings.

(2). Arbuscular mycorrhizal fungi are ubiquitous as to their choice of colonized host plant. Multiple studies have shown variations in effect among different mycorrhizal partners on various plant parameters. Such results lead to believe that the reduction of maize plant

TABLE 4.1: Examples WCR developing resistance to control methods

Year	Occurrence	Reference
1960's	WCR resistance to chlorinated hydrocarbon insecticides (aldrin and heptachlor)	(Ball and Weekman1962, Chio et al. 1979)
1970-80	Inconsistent efficiency of carbofuran	(Felsot et al. 1982)
1984	Inefficiency of isofenphos due to microbial degradation	(Felsot 1989)
1990's	Resistance to carbaryl and methyl parathion insecticides in Nebraska	(Meinke et al. 1998)
1980-90	WCR strain resistant to crop rotation in Illinois and Indiana	(Sutter 1999)

susceptibility to WCR infestation may also vary depending on the fungal element of the association. Important work therefore lies in the determination of optimal plant-fungus partner species in order to maximize beneficial effects on crops.

(3) Furthermore, a potentially important element to WCR control strategies is the reduction of both female fecundity and fertility. A negative mycorrhizal effect on the reproductive potential of the adults seems possible as a result of the stress that the insects appear to be subjected to at the larval stage.

(4) Finally, it is of prime importance to elucidate clearly the mechanisms underlying the mycorrhizal effects observed in our greenhouse trials if we are to utilize mycorrhizae most efficiently as important elements in WCR damage control and mediation.

CHAPTER V

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APPENDIX

Table A.1: ANOVA of mycorrhizal effect on plant parmaters: height, stalk circumference (SC), root dry (RDM) mass, shoot dry mass (SDM), and root damage (RD) for the 1998 greenhouse trial.

	df	Height		SC		RDM		SDM		RD	
Source		F	P	F	P	F	P	F	P	F	P
Inf	2	1.72	0.19	3.93	0.03	4.09	0.02	3.76	0.03	2.36	0.11
Myc	1	5.28	0.03	8.86	0.00	4.42	0.02	3.04	0.09	5.34	0.02
I*M	2	2.54	0.09	3.23	0.05	3.81	0.03	4.50	0.02	1.29	0.28

Sources of variation: Inf.= infestation treatment, Myc.= mycorrhizal treatment, I*M= interaction

Table A.2: ANOVA of mycorrhizal effect on plant parmaters: height, stalk circumference (SC), root dry (RDM) mass, shoot dry mass (SDM), and root damage (RD) for the 1999 greenhouse trial.

	df	Height		SC		RDM		SDM		RD	
Source		F	P	F	P	F	P	F	P	F	P
Inf	2	1.57	0.22	0.26	0.78	2.64	0.09	0.64	0.54	10.07	0.00
Myc	1	1.07	0..31	0.41	0.53	2.63	0.11	0.10	0.75	4.91	0.03
I*M	2	0.12	0.88	0.22	0.80	0.27	0.77	0.90	0.42	1.90	0.17

Sources of variation: Inf.= infestation treatment, Myc.= mycorrhizal treatment, I*M= interaction

Table A.3: ANOVA of mycorrhizal and infestation rate effects on emerging adult WCR head capsule diameter (H.C.D.) and mass (M) in 1999 greenhouse trial.

	df	H.C.D.		M	
Source		F	P	F	P
Inf	2	146.61	1.57	98.53	0.00
Myc	1	4.11	0.04	0.53	0.55
I*M	2	2.36	0.10	1.38	0.25

Sources of variation: Inf.= infestation treatment, Myc.= mycorrhizal treatment, I*M= interaction