1996

Interactions of vesicular arbuscular mycorrhizal fungi, herbicides and crops

Mercy Joseph Nedumpara
Iowa State University

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Interactions of vesicular arbuscular mycorrhizal fungi, herbicides and crops

by

Mercy Joseph Nedumpara

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Department: Agronomy
Major: Soil Science (Soil Microbiology and Biochemistry)

Approved:

Signature was redacted for privacy.

In Charge of Major Work
Signature was redacted for privacy.

In Charge of Major Work
Signature was redacted for privacy.

For the Major Department
Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1996
Dedicated to my parents
# TABLE OF CONTENTS

**GENERAL INTRODUCTION**..................................................................................1

Dissertation Organization.....................................................................................1

**LITERATURE REVIEW**.....................................................................................3

Introduction to Mycorrhizae.................................................................................3

Vesicular-Arbuscular Mycorrhizae.......................................................................4

The Rhizosphere....................................................................................................10

Plant-Herbicide Interactions.................................................................................12

Herbicide Behavior in Soil...................................................................................15

VAM-Herbicide Interactions.................................................................................16

**EFFECT OF A VA-MYCORRHIZAL FUNGUS (GLOMUS EPIGAEUS) ON HERBICIDE UPTAKE BY ROOTS**..............................................................................19

Abstract................................................................................................................19

Introduction............................................................................................................20

Materials and Methods........................................................................................21

Results and Discussion.........................................................................................25

Literature Cited......................................................................................................31

**IMPACT OF CORN AND A VA MYCORRHIZAL FUNGUS (GLOMUS EPIGAEUS) ON DEGRADATION AND UPTAKE OF ATRAZINE IN SOIL**.................................44

Abstract................................................................................................................44

Introduction............................................................................................................45

Materials and Methods........................................................................................46

Results and Discussion.........................................................................................50

Conclusions...........................................................................................................56

References.............................................................................................................56

**GROWTH RESPONSE OF MYCORRHIZAL AND NONMYCORRHIZAL CORN AND SOYBEAN TO TRIFLURALIN AND ATRAZINE**...........................................67

Abstract................................................................................................................67

Introduction............................................................................................................67

Materials and Methods........................................................................................69
GENERAL INTRODUCTION

The vesicular arbuscular mycorrhizal (VAM) fungi are root symbionts associated with many vascular plant species. The beneficial effect of VAM fungi on plant growth due to enhanced nutrient uptake has been well documented. Little is known about the effect of interactions among VAM fungi, crops and agrochemicals on plant growth. Carry-over of herbicide residues is a continuing field problem that interferes with the use of herbicide-sensitive crops in rotational and replant situations. The overall objectives of the study were to determine whether VAM fungi contribute to the uptake of herbicides by plant root systems and to determine the role of VAM fungi in rhizosphere detoxification of herbicides. In this research the uptake of two widely used herbicides atrazine and trifluralin by VAM-colonized and non-VAM roots of corn and soybean was studied. The direct role of VAM fungal hyphae on herbicide uptake was also examined. The potential role of VAM fungi in association with corn plants on degradation and bound residue formation of atrazine in soil was investigated. The influence of VAM-herbicide interactions on tolerance of soybean and corn to atrazine and trifluralin, respectively, at rates that simulate field carry-over was studied in greenhouse conditions. A better understanding of the interactions between VAM fungi, crops and herbicides may have implications in developing suitable herbicide-application strategies in protecting rotational and replant crops from herbicide injury.

Dissertation Organization

This thesis has been organized into chapters and contains three manuscripts prepared for publication. An introduction is followed by a literature review, the three manuscripts, general conclusions and references cited in the literature review.

The first manuscript is entitled "Effect of a Vesicular Arbuscular Mycorrhizal Fungus (Glomus epigaeus) on Herbicide Uptake by Roots." This research was to determine the contribution of VAM fungi on uptake of atrazine and trifluralin by corn and soybean root systems. The direct role of VAM hyphae on atrazine uptake was also examined. This manuscript is prepared for publication in Weed Science.

The second manuscript is entitled "Impact of Corn and a VA Mycorrhizal Fungus (Glomus epigaeus) on Degradation and Uptake of Atrazine in Soil." The effect of corn root systems colonized by Glomus epigaeus on degradation, bound residue formation, and plant uptake of atrazine, applied at field recommended rates was investigated. This manuscript is prepared for publication in the Journal of Environmental Quality.
The third manuscript is entitled "Growth Response of Mycorrhizal and Nonmycorrhizal Corn and Soybean to Trifluralin and Atrazine." This study was to determine the effects of VAM fungi on tolerance of soybean and corn grown in low and high P soils to herbicides atrazine and trifluralin, respectively, at rates that simulate herbicide carry-over. The effect of these herbicides on VAM fungal colonization of roots was also studied. This manuscript is prepared for publication in Agronomy Journal.
LITERATURE REVIEW

Introduction to Mycorrhizae

Mycorrhizae are indigenous to soils throughout the world. Mycorrhiza, meaning fungus root, was first recognized and described by Frank in 1885 while working on truffles in Prussia (Frank, 1885 as reported by Bagyaraj, 1991). He characterized the association as mutualistic because the sheath-forming fungus on the roots was not detrimental to the host tree and termed as ectotropisch (ectotrophic). Another type of association without compact sheath but with hyphal penetration within root cells was later described by Frank as endotropisch (endotrophic). Mycorrhizal fungi form symbiotic associations within the roots of an enormously wide variety of host plants. In this relationship, the fungus extends both into the host plant and into the surrounding soil. The relationship is described as a mutualistic symbiosis (beneficial to the plant and fungus) characterized by the flow of organic components from plant to fungus and inorganic components from soil through the fungus to plant.

Mycorrhizal associations are widespread geographically and among plant families and appear to have evolved and spread with the earliest land plants (Allen, 1991). The fossil records of one of the earliest known plants, *Rhynia*, had mycorrhizal-type structures resembling vesicles and arbuscules (Kidstone and Lang, 1921). The occurrence of mycorrhizae range from aquatic to deserts, low land tropics to high altitudes and many latitudes. It is generally accepted that there are only a few nonmycotrophic plant families (Gerdeman, 1968; Mosse, 1973). Despite the widespread occurrence and enormous impact on plant growth, little importance was given to mycorrhizae in ecological and evolutionary processes. Enormous research conducted on mycorrhizal fungi during the past few decades have shown that these fungi are key members of the soil microbiota that conduct activities which are crucial to plant establishment, development, nutrition and health.

Much of the mycorrhizal research so far was done on the role of mycorrhizae on nutrient cycling and plant growth. Now there is a shift in focus to other possible roles these fungi may play in the soil ecosystem such as developing soil structure, composition of microflora, soil binding and degradation of organic and inorganic pollutants in soil.
Mycorrhizal Types

The universality of mycorrhizal symbiosis implies the great diversity and ability of the fungi to establish a wide host range. The mycorrhizae have been broadly classified into five groups, primarily on the basis of morphology and anatomy, but also of either host plant taxonomy or fungal taxonomy (Bagyaraj, 1991; Reid, 1985). The different groups are briefly described in this paper. More emphasis is given to vesicular-arbuscular mycorrhizae (VAM), the subject of this dissertation.

Ectomycorrhizae (ECM) are formed by the higher orders of Basidiomycetes and Ascomycetes and a very large number of fungal genera have been identified in ectomycorrhizal association. About 3% of higher plants, mainly forest trees belonging to Fagaceae, Betulaceae, Pinaceae, Rosaceae and a few genera from other families form ectomycorrhiza. Ectomycorrhizae (septate or aseptate) form a structure called the mantle or sheath which encloses the rootlet. This network of hyphae extends into the root cortex and forms a complex intercellular system which appears as a network of hyphae between cells called the Hartig net. The hyphae on the root surface and those in the soil vary from short cystidia or setae to extensive rhizomorphs (Harley and Smith, 1983). Root growth patterns of the host plant are often altered by the ECM development on the root system, and the dichotomy or bifurcation of roots is recognizable without any special staining techniques.

The endomycorrhizae include three groups of mycorrhizae characterized by the intracellular growth in the root cortex (Bagyaraj, 1991). The first type is the ericoid mycorrhiza (Ericaceae) and the second type is the orchid mycorrhiza (Orchidaceae). These two have only a restricted host range. The third type is the most common endomycorrhiza in nature and known as vesicular-arbuscular endomycorrhiza (VAM). The fifth group is the ectendomycorrhiza. The Hartig net is well developed, but the ectendomycorrhizae generally lack the fungal sheath and the septate hyphae form intercellular coils.

Vesicular-Arbuscular Mycorrhizae

The vesicular-arbuscular mycorrhizae are the most wide spread group since they occur on a vast taxonomic range of plants. VAM are found in bryophytes, pteridophytes, gymnosperms and angiosperms which includes almost all cultivated crops, native grasses, herbs, shrubs, and majority of the forest and shade species (Gerdemann, 1968; Harley and
Smith, 1983). Unlike ECM there is no mycelial sheath formation and, more importantly, inter-intracellular penetration of cortical cells takes place.

**Anatomy and Morphology**

The vesicular-arbuscular mycorrhizal association has three important components, the root itself, the fungal structures within the cells of the root (intraradical phase) and extramatrical mycelium and spores (extramatrical phase) in the soil.

The initial infection of a root system involves a hypha encountering the root surface and penetrating the root either by direct penetration of epidermis by appressorium or by intercellular penetration and eventual cell wall penetration of a cortical cell in an internal position of the cortex (Sanders et al., 1975). The infecting hypha can arise from a germinated spore in the soil or extramatrical hyphae from infected roots. Then the internal spread of hyphae within the cortex and subsequent proliferation of hyphae to outside the root.

The internal morphology of VA mycorrhizae consists of the aseptate hyphae and the characteristic structures, the arbuscles which occur within the cortical cells, and vesicles which occur intracellularly and hence the name. The fungi colonize the epidermal and cortical cells but never invade the endodermis. The spread of the hyphae inside the root varies, depending on the plant and fungus involved. The hyphae may be entirely intracellular or mainly intercellular depending on the host species. For instance, *Endogone fasciculata* form almost entirely intracellularly on the tulip tree, whereas in maize it is primarily intercellular (Gerdemann, 1965). Morphologically, the extramatrical mycelium is continuous with the intraradical mycelium. The hyphae are aseptate in their active state, and colonize the outer cortical layer of roots. However septa may form when growing conditions are unfavorable or the fungus is dying. The size of the hyphae vary from 3 to 7 μm depending on the type of fungus involved (Abbot, 1982).

Vesicles are ovate to spherical bodies formed as an intercalary or terminal swelling of the hyphae of VAM fungi. Not all VA mycorrhizae form vesicles within roots. For example, *Gigaspora margarita* does not form vesicles but forms auxiliary cells, a group of vesicle-like structures. Vesicles are formed either intra- or intercellularly depending on the fungus and host species and the size vary from 30-100 μm diameter depending on the species. The vesicles may be thin walled or thick walled, resembling chlamydospores in most respects. Different hypotheses on the role of vesicles have been proposed. According to McLennan (1926) vesicles are storage organs, whereas some other authors
suggested that vesicles also function as reproductive organs (Bonfante and Banciotto, 1995).

Soon after infection VAM fungi produce a complex hyphal branching system within cortical cells called arbuscules. The ultimate branches of arbuscular hyphae are <1 μm diameter and the entire structure may fill the cells. From a functional point of view they are considered as the most significant structures as they serve as the site for fungus-plant metabolite exchange (Smith and Gianinazzi-Pearson, 1988). The arbuscules have only a short life span (4 to 5 days) and they are quickly digested and the contents are absorbed by the host.

The external structures of VAM fungi are the hyphae that grow outside the root into the soil (extramatrical phase) and the chlamydospores arising from these. The external hyphae are thin walled, or thick walled, with a striking variation in diameter, ranging from 2 to 27 μm (Nicolson, 1959). Of the diverse kinds of organisms found in soil, it is the mycorrhizal fungus, through its external hyphae, that provides a direct physical link between vegetation and the soil resources. Very little information is available regarding the amount and distribution of external hyphae in soils because of the difficulty in quantifying this phase. The development and spread of hyphae differs greatly according to plant, fungi and the soil type. Species of VAM fungi differ in the length of external hyphae produced at similar levels of infection. For instance, Abbott and Robson (1985) showed that *Glomus fasciculatum* produced almost ten times less external hyphae per cm infected root than by *Acaulospora laevis*, *Glomus tenue*, or *Gigaspora calospora*, despite a comparable levels of root infection. A wide range of external hyphal length has been reported from different field studies. Hyphal extension of up to 9 cm into soil and hyphal growth of 2.6-5.4 m g^{-1} soil have been reported by Miller and Jastrow (1994). Hyphal extension up to 11 cm into soil was seen by Jakobsen et al. (1992). In rye grass, the amount of external hyphae produced was almost 14 m g^{-1} soil compared to 730 mm root length g^{-1} soil (Tisdall and Oades, 1979).

The spores are large globose, elliptical to ovoid in shape, produced asexually on straight subtended hyphae and are called chlamydospores. Some VA mycorrhizae have an aggregation of spores in sporocarp. The size of the spores varies from 35 to 400 μm in diameter, and are thick walled, with a cytoplasmic content rich in oil globules.
Taxonomy of VA Mycorrhizae

The VAM fungi are classified according to morphological characteristics of the sporocarps or chlamydospores and hyphal attachment. These fungi belong to the family Endogonaceae of the order Endogonales, class Zygomycetes, subdivision Zygomycota. Seven genera have been recognized in Endogonaceae: Acaulospora, Sclerocystis, Endogone, Scutellospora, Gigaspora, Glomus, and Entrophospora (Hall, 1984). Of these only Endogone forms zygospores characteristic of Zygomycetes, while others probably lack sexual reproduction. Species of Endogone do not form VA mycorrhizal associations. Five genera commonly form endomycorrhizae, Glomus, Sclerocystis, Gigaspora, Acaulospora, and Scutellophora. Glomus is the genus used in the present study and a brief description of the genus is given here.

Glomus, the most common genus of VAM fungi, has over 50 species which form globose, ellipsoid or rather irregularly shaped spores that range from 20-400 μm. The spore walls can have one to many layers. The spores are thick walled (up to 30 μm), attached to a single subtending hypha, produced in the soil near plant roots, at the soil surface, or occasionally in roots, singly or in groups of a few to many, or in large aggregates called sporocarps. The spores of most species germinate by emergence of a germ tube directly through the subtending hypha or rarely through the spore wall (Hall, 1984).

Distribution of VAM in Ecosystems

VAM fungi are soil-borne, infect living plant roots, and exhibit little host specificity. The distribution of VAM species is influenced by a wide range of environmental, host plant and fungal effects and cultivation patterns. In general the spores germinate in accordance to favorable physical (moisture, temperature, etc.) and chemical (pH) environmental conditions. No additional host-plant stimuli are required for initiation of infection (Hetrick, 1984). The large spore size and energy contained therein may permit the germinated spores to grow through the soil for long distances in search of host roots. VAM fungi are primarily distributed vertically near the soil surface. VAM spores are mostly seen in the top 15-30 cm of soil, and their numbers decrease markedly below the top 15 cm (Readhead, 1977). However VAM can extend deep into the profile based on distribution of plant roots. Virginia et al. (1986) reported a high concentration of VAM spores at 4 m depth in a sand desert in a nutrient-rich zone immediately above the water table.
VAM fungi may be disseminated in a variety of ways. Active dispersal occurs as the mycelia grow through the soil from one plant root to another. Inter-plant bridges between roots formed by VAM fungi have been reported by Francis et al. (1986). Species of plant and VAM and root density may significantly influence the rate of VAM fungus spread and an efficient mycorrhizal fungus might move 0.43 m year\(^{-1}\) (Powell, 1979). The passive dispersal of spores or other propagules occurs by water, wind or animal vectors. VAM spores were identified in the digestive tracts or feces of soil animals like earthworms and ants (Bagyaraj, 1991; Gange, 1993).

**VA Mycorrhiza Effects on Plant Growth**

In general VA mycorrhizae have a favorable influence on plant growth, which is most apparent under suboptimal nutrition. Enhanced uptake of P by the fungal hyphae is the primary mechanism responsible for growth stimulation by mycorrhizae. The extent and activity of a plant root system will determine its ability to obtain nutrients from soil. The mycorrhizal hyphae extend several centimeters into the soil, beyond the root-depletion zone, extending the effective zone of uptake. Rhodes and Gerdemann (1975) observed that *G. fasciculatus* was able to transport P to mycorrhizal onions from as far as 7 to 8 cm from roots, thus greatly extending the effective zone of P uptake. Numerous reviews have discussed in detail the evidence which contributes to this conclusion (Sanders et al., 1975; Jeffries and Barea, 1994; Miller and Jastrow, 1994).

Many investigators have found that plants with VA mycorrhizae contain a higher concentration of P than non-mycorrhizal plants in soils containing low available P (Kothari et al., 1990b; Jakobsen et al., 1992; Khalil et al., 1994; Roldan-Fajardo, 1994). Bethlenfalvay et al. (1982) found that the greatest growth effect of VAM fungi occurred during the pod stage of soybean, when the concentration of available soil P fell below 10 ug g\(^{-1}\) soil, suggesting that the magnitude of the VAM effect was a function of P nutrition. However, large variation among VAM species occur in infectivity on host plant and effectivity on plant growth.

Enhanced uptake of several micronutrients has also been implicated in the VAM-plant response. Like P, most micronutrients have low diffusion coefficients and low solution levels and so uptake of these nutrients could be diffusion limited. Inoculation with mycorrhizae greatly improved growth and eliminated the deficiency symptoms due to low concentrations of N and other nutrients like Zn, S, Cu, Co, Fe, Ca and Mn (Rhodes and Gerdemann, 1978; Pacovsky, 1986; Kothari et al., 1990b; Sharma et al., 1992). It was not
clear whether the VAM effect was an indirect consequence of balancing P status of the plant. However, evidence of a VAM effect on uptake of micronutrients independent of a P response are also found in the literature. For example, hyphal transport of $^{35}\text{S}$ to mycorrhizal plants occurred when applied 8 cm away from the onion root and the nuclide was not present in non-mycorrhizal plants (Rhodes and Gerdemann, 1978). The exclusive uptake and translocation of $^{32}\text{P}$, $^{65}\text{Zn}$, $^{35}\text{S}$ by external hyphae of $G. \text{mosseae}$ was demonstrated by Cooper and Tinker (1978) and the P flux in the hyphae was estimated as $2 \times 10^{-10}$ mole cm$^{-2}$ s$^{-1}$. P flux of 0.8 to 3.2 nmol m$^{-2}$ s$^{-1}$ for hyphae of $G. \text{mosseae}$ was seen in studies by Smith et al. (1994). They suggested that the translocation in hyphae was an active process under metabolic control, influenced by the host P requirement.

**Mechanisms of Nutrient Uptake by VAM**

Different mechanisms of increased P uptake have been proposed by Sanders et al. (1975): (1) morphological changes in the plant, (2) provision of additional or more efficient absorbing surface in fungal hyphae with subsequent transfer to the host, (3) ability of the mycorrhizal root or hyphae to utilize sources of P not available to non-mycorrhizal roots, including absorption at lower solution concentrations, and (4) longer viability of mycorrhizal than of non-mycorrhizal roots.

The small diameter hyphae may explore smaller pore spaces than root hairs. It was also suggested that the high surface to volume ratio of VAM hyphae compared to roots make mycorrhizal roots more efficient for P uptake (O'Keefe and Sylvia, 1991). Sanders and Tinker (1973) reported a mean increase of 477% in P flow with a mean increase in surface area of only 3%. The large increase in P flow due to a small increase in surface area indicates that there is more to the mycorrhizal growth response than a simple increase in absorptive surface area. Root hair density, length and total root length are reduced in mycorrhizal plants (Sanders and Tinker, 1973, Kothari et al., 1990a). This suggests that the increase in P uptake by VAM cannot be attributed to an increase in root area alone, but also to a greater efficiency of the VAM fungal hyphal system for P absorption. In some instances, the uptake of P by mycorrhizal plants was 2 to 3 times faster than in uninfected plants (Karunaratne et al., 1986., Cress et al., 1979). The affinity (Km) of uptake was not increased in VAM soybean roots in studies by Karunaratne et al. (1986) and the authors proposed that the increased uptake was due to an increase in number of uptake sites per unit area of root (Vmax). Cress et al. (1979) reported a lower Km for P uptake in VAM tomato roots than in non-VAM roots, indicating an increased affinity of the absorbing sites.
in VAM roots. The mechanisms responsible for increased P uptake may also apply to the uptake of other diffusion-limited micronutrients.

Increased phosphatase activity by VAM fungi has been suggested as evidenced by the increased uptake of organic P forms like phytates (Jayachandran et al., 1992; Tarafdar and Marschner, 1994). Active phosphatases have been found in the internal hyphae of *Gigaspora margarita* (Becker & Hall) associated with onion roots (Saito, 1995). However, the role of phosphatase in VAM-mediated P uptake still remains unclear. Besides the direct VAM hyphal effects, alteration of root morphology, and root properties like membrane permeability and hydraulic conductivity by VAM colonization (Fiscus, 1975; Kothari et al., 1990a; Nelsen and Safir, 1982) may cause changes in the nutrient acquisition properties of roots.

### The Rhizosphere

The term rhizosphere comes from the Greek word 'rhizo' meaning roots and the volume of soil adjacent to that (sphere). The rhizosphere is the zone of increased microbial activity and biomass at the root-soil interface that is under the influence of the plant root (Curl and Truelove, 1986). The rhizosphere consists of three zones. The rhizosphere in the strict sense consists of the soil around the roots in which soluble and volatile compounds secreted by the roots diffuse. The rhizoplane, comprises the root surface and the mucigel covering part of the roots behind the root cap. The endorhizosphere consists of epidermis and cortex cells invaded by saprophytic microorganisms. The number of microorganisms in the rhizosphere (R) is generally much higher than in the soil (S) further away from the root. The R/S ratios commonly range from 5 to 20, but they can run as high as 100 or greater (Atlas and Bartha, 1992).

Rhizodeposition and root exudation are two means by which substrate is provided to the rhizosphere. Estimates for the annual amount of rhizodeposition and root exudates vary from 7 to 27% of the total plant mass (Moser and Haselwandtter, 1983). The different classes of materials released by roots include polymeric secretions (mucilages, enzymes), water soluble exudates, lysates, and gases like CO₂, ethylene, and terpenes (Foster, 1986). Root exudates include sugars, aminoacids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavones, enzymes, and numerous other compounds (Klein et al., 1990). Kraffczyk et al. (1984), reported that in sterile axenic culture the exudates from corn roots consisted of 65% sugars, 33% organic acids and only 2% amino acids. It was reported that soluble compounds constitute only a small fraction of the total plant discharge.
(Rovira and Davey, 1974). Gaseous products and non-soluble compounds of high molecular weight are quantitatively of much greater importance. The ratio between water soluble compounds, insoluble compounds, and volatile compounds has been estimated to be 1:3-5:8-10.

Exudation can occur over the entire length of the root, but the primary area of release is at the root tip. The composition varies and the amount of exudates in the rhizosphere increase in the presence of microorganisms, and also vary with nutritional status of the plant (Krafczyk et al., 1984). Increased exudates in nonsterile soil could be due to microflora altering the metabolism and permeability of roots (Linderman, 1994). The rate and composition of root exudates vary with the age and species of the plant, light, temperature, water, and competition. The release of compounds from roots depends on the concentration in roots and the permeability of roots.

Different plant species, even the same plant at different growth stages, support different flora depending on the exudation patterns by a complex interaction of growth enhancers, such as sugars, and inhibitors such as phenolic compounds. Rhizosphere exudations selectively enrich populations of some microbes while inhibiting others. The rhizosphere community consists of microbiota (bacteria, actinomycetes, fungi, protozoa, and algae) and mesofauna (nematodes, mites, and insects). Generally, the rhizosphere is colonized predominantly by gram negative bacteria (Atlas and Bartha, 1992). Bacteria live in colonies that may cover 4 to 10% of the root surface. Initial colonizers are often associated with soil organic matter.

Mycorrhizosphere

Mycorrhizosphere is based on the concept that mycorrhizae exert a strong influence on the microflora of the rhizosphere. Nutritional status of the host plant is altered by VAM colonization and also the large volume of fungus in the root tissue could affect the physiology of root and root exudation more directly. For instance, Dixon et al. (1989) have seen that colonization of Citrus sp. by Glomus sp. has caused significant change in the amino acid and reducing sugar content of the root exudates. Mycorrhiza formation may influence the rhizosphere microflora directly by their interaction with other organisms or indirectly by their effects on root exudation. The active and decaying external hyphae of mycorrhizal fungi and the exudates from the hyphae provide a physical or nutritional substrate for bacteria and other organisms (Paulitz and Linderman, 1991). This results in a shifts in the microbial populations that depend on roots for a source of energy.
Many researchers have demonstrated that differences in populations of taxonomic and functional groups of microorganisms occur between mycorrhizal and non-mycorrhizal plants. In the rhizosphere of mycorrhizal and non-mycorrhizal tomato (*Lycopersicon esculentum* L.) plants, greater populations of bacteria and actinomycetes occurred in the mycorrhizosphere, compared to the noninoculated control (Bagyaraj and Menge, 1978). Meyer and Linderman (1986) compared the qualitative selection of microbial population in the rhizoplane and rhizosphere soil of sweet corn (*Zea mays* L.) and subterranean clover (*Trifolium subterraneum* L.). There was no difference in total bacteria in the rhizosphere soils from mycorrhizal and nonmycorrhizal plants, but significant qualitative shift occurred. The facultative anaerobes increased in the mycorrhizosphere soil, but fluorescent *Pseudomonas* decreased, whereas, fluorescent *Pseudomonas* and the total bacterial population significantly increased in the rhizoplane of mycorrhizal plants compared to nonmycorrhizal plants. They also showed that the microbial community of the mycorrhizosphere adversely affected the sporangial induction of the root pathogen *Phytophthora cinnamomi* (Rands.). Apparent selection of morphologic and physiological groups of bacteria and population change between VAM and non-VAM rhizospheres have been reported in studies by Ames et al. (1984) and Secilia and Bagyaraj (1987). VAM fungi have been shown to alter plant root concentration of antimicrobial compounds like phytoalexins and associated isoflavonoids (Morandi et al., 1984). It can be concluded from these reports that mycorrhiza formation in plants can alter the microbial make up of the rhizosphere and thus influence the utilization of various organic substrates in the rhizosphere.

**Plant-Herbicide Interactions**

Herbicides fall into two major groups, selective (kills a particular range of plants) and non-selective (total vegetative control). The major classes of chemicals used in weed control are: triazines, amides (haloacetamides), benzoics, bipyridiliums, carbamates, dinitroanilines, ureas, phenoxy (hormones), biphenylethers, nitriles, thiocarbamates, uracils and other unclassified types. In this review emphasis is given for herbicides atrazine, 6-chloro-N-ethyl-N-(1-methylethyl)-1,3,5-triazine-2,4-diamine (triazine) and trifluralin, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzamine (dinitroaniline) since these two chemicals are used in the present study.

The absorption and translocation of a compound by plant tissues depends on its molecular configuration, plant characteristics, and environmental conditions. Herbicides
enter into roots by three routes such as apoplast, symplast, and apoplastic-symplast depending on the physical and chemical properties of the compound. The apoplastic route involves movement exclusively in cell walls to the xylem. The symplastic route involves entry into cell walls and sequential passage into the endodermis, stele, and phloem. Under most conditions, there is rapid translocation upward from roots in the xylem by transpirational stream but only limited upward transport in the phloem (Ashton and Crafts, 1981).

The uptake of organic substances by plant roots occurs in two phases (Peterson and Edington, 1976). First is the partitioning onto the external surfaces and rapid accumulation of chemical into the free space of the roots. The second, superimposed on the first, is a slow continuous active (requires energy) uptake into less accessible tissues of the root, xylem or passage across membranes (plasmalemma) into the symplasm. Most herbicides move across plasma membranes via nonfacilitated diffusion because the membrane’s lipid bilayer is permeable to neutral, lipophilic xenobiotics. Experimental evidences suggest that the herbicides atrazine (triazine) and trifluralin (dinitroaniline) are readily absorbed into tissues across the plasma membrane by passive diffusion and reach an equilibrium with the external solution (Boulware and Camper, 1973; Darmstadt et al., 1984; Price and Balke, 1983). Passive absorption of lipophilic, ionic herbicides or weak acids can be mediated by an ion-trapping mechanism where the less lipophilic, anionic form accumulates in alkaline compartments of the plant cell (Sterling, 1994). The rate of movement of nonionic organic compounds into roots can be related to the octanol partition coefficient ($K_{ow}$) of the compound. Compounds with low $K_{ow}$ (<2) are likely to have faster transport into root tissues (Ryan et al., 1988).

**Mode of Action of Atrazine**

Atrazine is commonly applied as a pre-emergent herbicide in soil. Root uptake of atrazine readily occurs in plants whether resistant or sensitive to the herbicide in proportion to the concentration and time of exposure. All triazine herbicides partition exclusively into chloroplast. The site of inhibition is a 32 kD polypeptide (QB) which forms part of the Photosystem II located within the thylakoid membrane of the chloroplasts (Gardner, 1981). When they bind to D1 proteins, shunt electrons formed by the Hill reaction (photolysis of water) into the chloroplast stroma. These electrons form highly reactive free radicals in the chloroplast, such as super oxide. These unstable free radicals oxidize membranes and photosynthetic pigments resulting in chlorosis.
Mode of Action of Trifluralin

Trifluralin is a dinitroaniline group herbicide. It is a pre-plant herbicide incorporated into soil. Trifluralin is absorbed both by roots and shoot of germinating seedlings. Trifluralin inhibits cell division in the meristematic tissue by binding to the protein tubulin, which in turn prevents tubulin assembly into microtubules (Hess, 1987). Microtubules are required for cell division and cell wall formation. Thus, trifluralin disrupts the mitotic process, preventing cell wall formation, and causing enlargement of cells and extensive replication of nuclei. In general, it limits root growth, especially the development of lateral roots. Often only the primary roots develop and these are somewhat thickened and stubby, especially at the tip, and have only a limited number of secondary roots. Even though major symptoms appear on the root, associated effects are also possible at other parts of the plant.

Mechanisms of Atrazine Resistance

Different theories were attributed for atrazine resistance. Mechanisms which confer atrazine resistance include a change in the binding site of the herbicide or a reduction in the concentration of the herbicide at the binding site. A change in binding site involves a change in structure and composition of the binding site, the 32 kD polypeptide within QB protein. The amino acid serine is substituted by glycine in the polypeptide in resistant biotypes which may cause a change in affinity of the binding site for the herbicide (Hirschberg and McIntosh, 1983). An alternate mechanism for triazine resistance is the reduction in concentration of herbicide at the binding site by detoxification. Corn plants resistant to atrazine possess enzymatic and non-enzymatic detoxification mechanisms (Shimabukuro, 1967; Shimabukuro et al., 1970, 1971). The enzymatic mechanism involves the synthesis of a conjugate between glutathione and atrazine catalyzed by glutathione s-transferase (GST). Conjugate formation by GST was found to be the predominant mechanism for atrazine detoxification in corn and sorghum when the compound was directly introduced into the leaves. The enzyme is located mainly in leaves and roots contain no detectable activity.

Degradation by hydroxylation (to hydroxy atrazine) or dealkylation was also considered to be of importance in atrazine resistance. A number of studies have demonstrated that the non-enzymatic hydroxylation is a primary degradation pathway in corn (Shimabukuro, 1967; 1968). Oxidative N-dealkylation and deamination of atrazine play an important role in selectivity, especially in species with intermediate tolerance like
cotton. Not all corn cultivars are atrazine tolerant and the susceptibility of line GT112 has been attributed to a low GST (Shimabukuro et al., 1971).

Mechanisms of Trifluralin Resistance

Tolerance to trifluralin is expressed by resistance to the biochemical effects of the herbicide, degradation by plants and alteration in the herbicide site of action. Trifluralin is poorly metabolized by higher plants (Probost et al., 1967) and hence degradation by plant is not a significant resistance mechanism. Metabolites of trifluralin are not toxic like the parent compound. The resistance to the biochemical effects of trifluralin is considered as the major resistance mechanism. In many instances, resistant biotypes were less sensitive to trifluralin due to alteration of the binding protein tubulin (Vaughn, 1986). The target protein, tubulin, is altered and microtubule formation and cell division are not inhibited.

Herbicide Behavior in Soil

Fate of herbicides in soil is influenced by several factors such as moisture, temperature, aeration, soil chemical properties, microbial activity and physico-chemical properties of the compound. The pesticide undergoes complex interactions in soil leading to sorption or breakdown of the compound by biotic and abiotic processes. Immobilization by bound residue formation (fraction remaining after exhaustive solvent extraction) is often considered in the fate of pesticides in soil. Binding of pesticide to form humified complexes is mainly biological by enzymatic activity (Katan and Lichtenstein, 1977), even though abiotic binding is also possible. The availability of bound fraction in soil for further transformations is not clear.

Atrazine in Soil

Sorption and degradation are the key processes controlling the persistence and leaching of atrazine in soil. Atrazine is readily sorbed by a variety of clay minerals and organic matter (Francioso et al., 1992) which reduces the availability for leaching and degradation. Atrazine is degraded in soil by either microbial or chemical means (Skipper and Volk, 1972; Winkelman and Klaine, 1991). Microbial degradation is generally characterized by N-dealkylation and chemical degradation by hydrolysis. The major atrazine degradation products in soil are deethylatrazine (DEA), deisopropylatrazine (DIA), dealkylatrazine (DAA), hydroxyatrazine (HYA) and deethyldeisopropylatrazine (DEDIA). Loss from soil by volatilization was measured as low in field studies (Whang, 1993).
half life of atrazine in soil varied from less than a week to months (Marriage et al., 1975; Keller and Weber, 1995).

Atrazine is moderately persistent in soil and residues generally do not remain in soil for more than one year. However, carry-over residues of atrazine were seen in field studies and sometimes at levels to cause potential yield reduction to sensitive crops especially after successive applications of the herbicide (Burnside et al., 1971; Marriage et al., 1975). For instance, field studies conducted by Marriage et al. (1975) have shown that low concentrations of atrazine (0.4 kg ha⁻¹) present in soil from previous applications caused 38% yield reduction in oats (Avena sativum L).

Trifluralin in Soil

Trifluralin disappearance from soil includes physical loss by volatilization, sorption and degradation through photochemical, microbiological and chemical processes. The sorption constant (Koc) for trifluralin is 10 times or more than that of atrazine (Francioso et al., 1992). Short range diffusion may be important for herbicide efficacy or loss from the soil surface. Both solution and vapor-phase-transport components affect trifluralin diffusion. Phytotoxicity is apparently related with adsorption strength and volatility. Trifluralin is degraded by aerobic and anaerobic degradation pathways and the metabolites are numerous (Probst et al., 1967). Aerobic degradation proceeds through a dealkylation followed by a reduction while anaerobic degradation occurs with a preliminary reduction prior to dealkylation.

In general trifluralin is moderately persistent in soil and the half life of trifluralin varies from weeks to months in different soils. For normal use rates, persistence in soil ranges from 5 to 6 months. Persistence increases with decreasing soil temperature and moisture content. In a field study conducted by Smith (1972), 10 to 20% of trifluralin applied in soil at normal rates, remained after 5 months. Carry-over of trifluralin residues at phytotoxic levels has been reported occasionally when applied at normal and above normal rates or after high frequency of application (Raulston et al., 1971; Miller et al., 1975).

Herbicide-VAM Interactions

The effect of agricultural chemicals on VAM is poorly understood and pesticide application may have inadvertent or unrecognized effects. Observations of pesticide effects on VAM fungi are generally limited to measures of sporulation or root colonization. Herbicides can affect VA mycorrhizal fungi directly or indirectly through their effects on host
plants. In general, herbicides appear to have little direct effect on VAM fungi. Host plants may often be more sensitive to a herbicide than their mycorrhizal fungi, which is particularly true of the photosynthesis-inhibiting compounds.

Experimental evidence suggests that, in many situations, the herbicide concentrations in soil treated at recommended rates are much lower than concentrations required to cause toxicity and have little effect on VAM symbiosis or function. In some instances, herbicides which are nontoxic at low concentrations are toxic at higher concentrations. For example, alachlor and trifluralin applied at recommended rates (2 kg ha\(^{-1}\)) to soybean did not appear to have a direct effect on root growth and VAM fungal colonization of soybeans (Burpee and Cole, 1978). When alachlor was applied at 4 kg ha\(^{-1}\), both soybean root growth and fungus hyphal elongation were reduced. The effect on the hyphae persisted after the roots had recovered from inhibition, indicating a direct effect on the fungus. In the same study trifluralin did not have any adverse effect above the recommended rates. Smith et al. (1981) found that diuron and trifluralin at field-recommended rates had minimal effects on VAM development in wheat. In another study, Nemec and Tucker (1983) also showed that trifluralin and diuron had little adverse effect on VAM formation or growth of Citrus spp., but simazine and paraquat mixture applied at moderate rates reduced VAM formation. The toxicity to VAM appeared as a result of herbicide toxicity in plants as evidenced by the reduced growth parameters. The widely used triazine herbicides were generally not harmful to VAM formation in plants. Atrazine did not affect VAM formation in Liquidambar styraciflua (Trappe et al., 1984). Simazine did not affect root colonization by Glomus versiforme in apple and hyphal elongation in invitro studies (Hammel et al., 1994). In the same study, dichlobenil and paraquat did not affect root colonization but reduced hyphal elongation in invitro studies. Garcia-Romera and Ocampo (1988) found that 120 mg kg\(^{-1}\) soil of MCPA [4-chloro-2-methylphenoxy]acetic acid] was required to lower colonization by Glomus mosseae in pea roots while indigenous species showed susceptibility at much lower doses.

Herbicides applied at field recommended rates have caused reduction in VAM colonization. In a study by Dodd and Jefferies (1989), colonization of wheat by Glomus monosporum was reduced by a mixture of mecoprop, ioxynil and clopyralid, and bifenox applied alone while colonization by two other Glomus spp. was not consistently affected. No herbicide damage to wheat was evident suggesting a direct toxicity to VAM colonization. In a greenhouse study, oxifluorfen and oxadiazon reduced root colonization of cassava (Manihot esculenta) (Sieverding and Leihner, 1984).
Effect of herbicides on spore production and germination was also examined in the above studies. Dodd and Jeffries (1989) showed that low concentrations of a herbicide mixture containing bifenox, mecoprop and another mixture containing mecoprop, ioxynil, and clopyralid inhibited spore germination of *Glomus spp.* while higher concentrations were stimulatory. They also observed that herbicide toxicity to spore production by *Glomus spp.* varied with VAM species. This results suggests that herbicides selectively inhibited certain species and thus affected species composition.

Another significant aspect of VAM interaction was its effect on crop response to herbicides. Limited studies have shown that VA-mycorrhizal associations increase or decrease crop tolerance to herbicides. Increased damage to VAM soybeans from atrazine residues (Busse and Ellis, 1987) and to VAM apple from herbicides paraquat, simazine and dichlobenil (Hammel et al., 1994) have been reported. The increased crop injury in VAM plants was postulated to the VAM-mediated uptake of herbicides. This hypothesis was supported by the finding of Nelson and Khan (1992) that hyphae of *G. mossae* were able to remove atrazine from the soil and transfer to corn plants. In some other studies, VAM colonization reduced crop injury from herbicides imazaquin, carbamate herbicides, cyanazine and MCPA (a chlorophenoxyacetic acid herbicide) when applied at low or intermediate doses (Garcia-Romera and Ocampo, 1988; Garcia-Romera et al., 1988; Siqueira et al., 1991). However, any herbicide-safening mechanism of VAM fungi in plants has not been reported. The effectiveness of VAM fungi in improving herbicide tolerance varied with different endophytes. In most cases, the alleviation of herbicide phytotoxicity was attributed to the growth promotion by VAM fungi due to increased P uptake.

In agricultural soils where the mixed populations of VA-mycorrhizal fungi normally occur, the side effects of pesticides on these non-target microorganisms are very complex. Species of VAM fungi differ in their response to a particular chemical, so no generalization can be made on the toxicity of a chemical to VAM fungi. More research is needed to better understand the different aspects of VAM-herbicide interactions. This information will help to develop safe herbicide application strategies to preserve the symbiosis, in situations where mycorrhiza formation is considered to be important.
EFFECT OF A VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS (GLOMUS EPIGAEUS) ON HERBICIDE UPTAKE BY ROOTS

A paper to be submitted to Weed Science
Mercy J. Nedumpara, Thomas B. Moorman, and Krish Jayachandran

Abstract: Experiments were conducted to determine the influence of vesicular-arbuscular mycorrhizal (VAM) fungus on root uptake of herbicides. Absorption of atrazine by excised roots of corn and soybean colonized with and without the VAM fungus, Glomus epigaeus (Daniels & Trappe) from herbicide solutions was compared. Similarly, trifluralin uptake by excised VAM and non-VAM soybean roots was studied. Herbicide uptake was consistently greater by VAM roots. Atrazine and trifluralin uptake from herbicide solutions were also studied by using whole-root systems of soybean and corn, respectively, with and without VAM fungus. Atrazine and trifluralin uptake by intact root systems were enhanced by VAM association, however increase in uptake by mycorrhizal roots was more evident in herbicide absorption by excised roots. The direct role of VAM hyphae on atrazine uptake was studied by using a two-chamber system where only the fungal hyphae had access to the $^{14}{\text{C}}$-atrazine-treated soil. Hyphal systems of the fungus were able to remove and transfer $^{14}{\text{C}}$-atrazine residues from soil to corn plants. Our results suggest that the VAM association enhances the uptake of atrazine by corn and soybean roots as influenced by the direct uptake of the herbicide by the fungal hyphae. Nomenclature: atrazine, 6-chloro-N-ethyl-N’-(1-methylethyl)-1,3,5-triazine-2,4-diamine; trifluralin, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzamidine; corn, Zea mays L.; soybean, Glycine max (L.) Merr.

Additional index words: VAM fungi, Glomus epigaeus, excised roots, external hyphae, atrazine, trifluralin.

1Received for publication and in revised form. Joint contribution of USDA-Agric. Res. Serv. and Iowa State Univ. Journal Paper No. of the Iowa Agric. and Home Econ. Exp. Stn., Ames, IA 50011.
3Microbiologist, USDA, Agric. Res. Serv.
INTRODUCTION

Atrazine and trifluralin are two widely used herbicides for the selective weed control in the cultivation of many agronomic crops. The rate and extent of removal of these chemicals by plant root systems have economic and environmental significance. Information on VAM-mediated uptake of pesticides by plants may have implications for several agronomic and environmental issues. VA mycorrhizal fungi are indigenous to most soils throughout the world, and they occur on a vast taxonomic range of plants. If VAM can contribute to pesticide uptake by target plants, it improves pesticide efficacy. In replant situations following crop failure, however, increased herbicide uptake may lead to added injury when a herbicide-sensitive crop substitutes for a tolerant crop. The enhanced removal of carry-over residues of pesticides by mycorrhizal root systems may influence the fate of pesticides in the environment. Also in phytoremediation approaches, mycorrhizal root systems may be more efficient for the on-site cleanup of contaminated soils (7).

Several studies have investigated the absorption and translocation of a number of herbicides by various excised and intact root systems without confounding effects of environmental conditions (17, 18, 26, 32). Triazine herbicides, including atrazine, are readily absorbed by excised roots of different plants (6, 24, 28, 29). In general, passive absorption of herbicide into root tissues occurs and reaches an equilibrium with the external solution. In tissue tests using excised roots, the energy gradient created by transpirational flow is lacking, which enables a better understanding of the potential of root absorption and accumulation of organic compounds. However, information on the absorption of herbicides by excised root systems colonized with VAM fungi is lacking.

Limited investigations conducted on the role of VAM fungi on the uptake of organic pesticides uptake have shown that these fungi are able to absorb organic compounds from soil and transfer them to plants. Increased uptake of $^{14}$C-fonofos (O-ethyl S-phenyl ethylphosphonodithioate) residues from soil by onion (Allium cepa L.) roots colonized by Glomus sp. was reported by Nelson and Khan (21). They found that VAM fungal hyphae were able to absorb and translocate soil-bound residues of $^{14}$C-fonofos to plants. Hyphal uptake and transport of $^{14}$C-atrazine to corn by Glomus spp. were also demonstrated by Nelson and Khan (22).

The overall objective of this research was to determine the contribution of VAM fungi to herbicide uptake by plant root systems. Atrazine and trifluralin are widely used in corn and soybean crops, respectively, and VAM formation in these crops has been well
documented (13, 15). In this study, experiments were designed to compare the short-term rates of atrazine and trifluralin uptake by excised and intact root systems of com and soybean colonized with *Glomus epigaeus* and without the fungus. The direct exposure of different root systems to pesticide solutions gives a comparison on the efficiency of root uptake by eliminating the influence of other environmental factors. The direct role of VAM fungal hyphae in herbicide uptake was also examined by using a specially designed experimental system.

**MATERIALS AND METHODS**

*Herbicide Uptake by Excised Roots*

**Plant growth.** Soil used for the different experiments was collected from the borders of long-term fertility plots at the old Agronomy Farm of Iowa State University, which were maintained at low available P (<15 mg kg\(^{-1}\)) status. A soil:sand mixture (1:1 by vol) was prepared, and the available P (Bray 1) content of the mixture was 10 to 16 mg kg\(^{-1}\) soil. The soil-sand mixture was sterilized by autoclaving two times at 121 C, with 24 h duration between sterilization cycles. Com and soybean were grown in 3 kg (oven-dry basis) of sterile soil-sand mixture inoculated with *G. epigaeus* at 500 spores kg\(^{-1}\) soil or without the VAM inoculum. Soil (6 to 8 g) containing VAM fungal spores and fungal hyphae from asparagus (*Asparagus officinalis* L.) pot culture was used as the VAM inoculum. Microbial inoculum was prepared by blending 100 g of field soil with 1 L of water. The soil in the slurry was allowed to settle and the supernatant was passed through a 38-μm sieve to remove the VAM fungal inoculum. The filtered solution was added at 100 ml pot\(^{-1}\) to re-establish the heterotrophic population of bacteria and fungi removed by autoclaving.

Soybean variety Wells-11\(^{5}\) and corn variety 8543\(^{6}\) were used for the study. Corn and soybean plants were grown in greenhouse conditions equipped with auxiliary lighting (400-W, high pressure Na lamps) in a completely randomized design. The plants were fertilized with 50 ml of Hoagland's solution without P (11) at weekly intervals and were grown in a 13-h photo period at a mean day temperature of 27 C.

**Herbicide absorption.** Atrazine absorption experiments were performed using corn grown for 6, 7, and 8 wk and soybean grown for 6 and 7 wk. The trifluralin absorption assay was conducted using soybean grown for 7 wk. Shoots were removed and roots were carefully

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\(^{5}\)Purdue Univ., West Lafayette, IN.

\(^{6}\)ICI Corp., Slater, IA.
washed by soaking in water contained in large buckets to remove soil and minimize the
breakage of external hyphal network. The fine, tertiary roots were sampled at random, cut
into 15-to 20-cm segments and divided into 100-cm samples, as determined by the line-
intersect method (9). The root segments were stored in buffer solution approximately at pH
7 consisting of 0.5 mM KCl, 0.25 mM CaSO₄, 25 mM tris (hydroxymethyl) aminomethane
(Tris), and 25 mM of 2-(N-morpholino) ethanesulfonic acid (Mes) until treatment with the
herbicide (28).

Herbicide absorption by excised roots from VAM infected or non-VAM soybean and
corn plants was studied by the method described by Price and Balke (28). Stock solutions
of atrazine and trifluralin at 0.7 and 7-μM concentrations containing unlabeled and ¹⁴C-
labeled herbicide were prepared in buffer solutions. Atrazine (>99% purity)⁷ and [U-¹⁴C-
ring]-atrazine (radiochemical purity >98%)⁸ were dissolved in methanol and 0.7 and 7-μM
concentrations were prepared by dilution in buffer solution. Similarly, stock solutions of
trifluralin (99% purity)⁹ and [U-¹⁴C-ring]-trifluralin (radiochemical purity >99%)¹⁰ were
prepared and diluted with buffer to 0.7 and 7-μM concentrations. The ¹⁴C-activity of the
solutions was 0.002 μCi ml⁻¹ at both concentrations of the herbicides. Roots were placed
in glass test tubes containing 10 ml solutions of 0.7 and 7 μM atrazine or trifluralin. Root
samples were incubated in buffer solution containing the herbicide for 30, 120, 240, 360,
and 1440 min. Each treatment was replicated three times. At the end of the absorption
period, the treatment solutions were drained off and roots were washed for 1 to 2 min in
cold herbicide-free buffer solution to remove herbicide adsorbed on cell wall and diffused
into the apoplast. Experiments conducted with rinsing of the roots using buffer solution
containing unlabeled atrazine or trifluralin did not alter the removal of labeled herbicides
from roots compared to rinsing with herbicide-free buffer solution (data not shown).
Therefore unlabeled atrazine was not included in the wash solution. Similar observations
were made by Price and Balke (28). The roots were air dried and the herbicide uptake was
quantified by measuring the radioactivity in roots. The roots were combusted in a biological
oxidizer¹¹ and ¹⁴CO₂ evolved was collected in a special scintillation cocktail¹². The ¹⁴C-
activity trapped in the cocktail was measured as dpm (disintegrations per minute) by liquid

⁷Chem Service, West Chester, PA.
⁸Sigma Chemical Co., St. Louis, MO.
⁹Chem Service, West Chester, PA.
¹⁰Sigma Chemical Co., St. Louis, MO.
¹¹R. J. Harvey Instruments Corp., NJ.
¹²Harvee ¹⁴Carbon cocktail.
scintillation spectrometry\textsuperscript{13}. The uptake of herbicide was calculated from the radioactivity recorded as dpm for the sample and the specific activity of $^{14}$C-herbicide in the buffer solution used for incubation of roots.

**VAM colonization.** Subsamples of the roots used in absorption assays were taken at random for measurement of VAM colonization. The roots were cleared and stained in 0.025\% trypan blue (27). Total and VAM-infected root lengths were measured using the line-intersect method (9). The VAM root colonization was calculated as the percentage of root length containing internal evidence of mycorrhizae (hyphae with arbuscules or vesicles). The development of external hyphae was also examined microscopically, but we were unable to quantify the external hyphal length since the hyphae occurred as entangled masses.

**Herbicide Uptake by Intact Root Systems**

**Herbicide absorption.** Short-term rates of herbicide uptake by intact VAM and non-VAM root systems in herbicide solutions were compared. Atrazine uptake by soybean roots and trifluralin uptake by corn roots were measured. Corn and soybean plants were grown in sterile soil containing 11 mg kg\textsuperscript{-1} P (Bray 1), inoculated with and without the VAM fungus. Soil preparation, VAM inoculation, and plant growth conditions were the same as described before. Corn and soybean were also grown in sterile soil containing additional available P to obtain growth comparable to mycorrhizal plants. An additional 60 mg P kg\textsuperscript{-1} soil (KH$_2$PO$_4$ solution) was added to low P soil in equal doses at planting and 15 d after planting. Intact root systems were collected with minimum disturbance to external hyphal network using the procedures described in herbicide uptake by excised roots. The root systems were placed in 1 L of buffer solution at pH 7 (28) with herbicide at 7-\textmu M concentration containing 0.045 \textmu Ci L\textsuperscript{-1} of $^{14}$C-herbicide. The roots were incubated in the herbicide solutions for 24 h, and washed in cold, herbicide-free buffer solution. After drying and grinding, a known weight was combusted as described in the excised root study to determine the $^{14}$C absorbed. The herbicide uptake per-gram dry weight of the root was calculated from the radioactivity measured for the sample and the specific activity of $^{14}$C-herbicide in solution used for incubation of roots. VAM colonization of the samples was also measured as described in the excised root absorption study.

\textsuperscript{13}1600 Packard Scintillation Counter, Packard Instrument Co., Meriden, CT.
Herbicide Uptake by VAM External Hyphae

Treatment vessels. Atrazine uptake in VAM and non-VAM corn was compared using plants grown in special two-compartment containers designed to allow hyphal penetration (without roots) into a compartment containing herbicide-treated soil. Movement of the herbicide into plant would constitute direct evidence of hyphal uptake and translocation from soil. Experimental vessels were constructed using 20-cm diameter, clear acrylic pipe to form two compartments. The two compartments were separated by a 400-μm metal (galvanized iron) screen (44 mesh) and a 40-μm (330 mesh) nylon screen. The metal screen was to support the soil in the top compartment without contacting the herbicide-treated soil in the bottom compartment. The nylon screen prevents root entry, but allows hyphal penetration to the bottom compartment containing 14C-atrazine. Sterilized soil (1.65 kg) was treated with atrazine at 1 mg kg⁻¹ soil containing 0.6 μCi of 14C-atrazine kg⁻¹ soil. Herbicide stock solution was prepared by dissolving labeled and non-labeled atrazine in methanol. Sterile soil was placed in layers in a special cylindrical mixing vessel and atrazine dissolved in methanol was applied as fine drops to each layer. The mixing container was then placed on a belt roller which turned the container to provide a thorough mixing of the 14C-herbicide with the soil. The bottom cell was glued to a plexiglass sheet on the lower side and then filled with the atrazine-treated soil. The soil was moistened and allowed to settle and approximately 2 mm of space remained above the soil. Then the metal screen and nylon screen were glued to the top of the bottom cell. The gap between the screen and the bottom soil was to prevent direct soil contact and water movement between the two compartments. The top cell was attached over the nylon screen. Sterile soil (5 kg) inoculated with G. epigaeus at 500 spores kg⁻¹ soil or without the VAM inoculum was placed in the top compartment and corn was grown. The experimental design was a completely randomized design with four replications.

Plant growth. The plants were grown in a growth chamber under a 13-h photo period provided by artificial lighting (400-W, high pressure Na lamps). The mean minimum and maximum temperatures were 20 and 30°C, respectively, and the relative humidity was 60%. Soil water content was maintained approximately at field capacity. Watering was restricted to the top compartment by visual observation of the water movement through the walls of the clear pipe. Infiltration was allowed to the bottom of the top cell to promote growth of roots over the screen and to facilitate VAM hyphal penetration from the infected

14Tetko, Inc., NJ.
15Bailey MFG., Inc. IA.
root mat into the bottom compartment containing herbicide-treated soil. Seventy-five milliliters of Hoagland's nutrient solution without P was applied at weekly intervals.

**Plant harvest.** The plants were harvested 8 wk after planting, which allowed a growth period sufficient for the development of external VAM hyphae. The shoot and leaf portions were harvested separately. The soil from the bottom chamber, 1 to 2 cm immediately below the screen, was sampled for estimation of VAM fungal development. The plant materials were oven dried at 50 C. The amount of \(^{14}C\)-herbicide uptake in plants was determined by powdering plant samples, combustion, and liquid scintillation counting. Atrazine concentrations were calculated from radioactivity measured on dry-weight basis and using specific activity of \(^{14}C\)-atrazine applied to soil. Tissue concentrations of \(^{14}C\) residues were calculated based on the assumption that atrazine remained as parent compound. The presence of metabolites would result in estimated concentrations slightly greater than the actual concentrations.

**VAM spore estimation.** Chlamydospores of *G. epigaeus* present in the soil were determined by wet sieving of the soil (5) using a nest of sieves (423 to 38 \(\mu m\)). The spores retained on the sieves were pooled together and systematically counted under a stereo microscope at 40X magnification.

**Data analysis.** Rate constants for herbicide uptake by excised roots were determined by non-linear regression techniques using a modified first-order equation, absorption \((A) = So(1 - e^{-kt}) + (k_0t)\), where \(So\) is the maximum initial uptake (pM), \(k\) (h\(^{-1}\)) is the uptake rate constant describing the initial uptake process (initial rate), and \(k_0\) (pM h\(^{-1}\)) is the parameter describing the rate of uptake during the later part of the reaction, and \(t\) is time (min). The regression analysis was done using SAS\(^{16}\). In whole root uptake and hyphal uptake studies, the results were analyzed by analysis of variance and LSD tests for mean comparisons using SAS.

**RESULTS AND DISCUSSION**

**Herbicide Uptake by Excised Roots**

**Effects of atrazine concentration and time.** Absorption of atrazine by both mycorrhizal and nonmycorrhizal soybean and corn roots was greatly dependent on herbicide concentrations (Figures 1 and 2). Atrazine absorption at 7 \(\mu M\) was generally 8 to 11 times

\(^{16}\)SA Institute Inc., Cary, NC.
greater than the uptake at 0.7 μM for both VAM and non-VAM roots after a 1440 min exposure, which indicates a direct relationship between uptake and concentration.

The root tissue rapidly absorbed atrazine, accounting for 12 to 79% of the total uptake during the first 30 min (Table 1). The rapid influx was followed by a period of slow uptake in some treatments. This biphasic pattern of absorption was also described in previous reports on the absorption of atrazine and other herbicides by different tissues of several plant species (6, 17, 19, 24, 28, 29). Other studies on atrazine uptake using excised soybean and corn root tissues showed that 40% and above of the total atrazine absorption took place during the first 5 to 10 min in a 30-min incubation period (6, 19, 29). Root absorption of solutes may be partitioned into apoplastic (the volume external to cell membranes) and symplastic components. The apoplast constitutes approximately 20% of the tissue volume. In our experiments, the short rinse of the roots with atrazine-free buffer was done to exchange the 14C-atrazine absorbed in the free space (apoplastic space), thereby minimizing the apoplastic fraction of uptake. Price and Balke (28) reported that a short rinse with cold absorption solution removed up to 30% of atrazine absorbed, an amount approximately equal to the apparent free space and cell wall volume. Studies by Price and Balke (28) and Peterson and Edgington (26) indicated that atrazine uptake is not strictly an apoplastic process, but the herbicide penetrates readily into the symplast.

Tissue accumulations of atrazine were much higher than the concentrations that could possibly be retained in the apoplastic volume during a passive equilibrium with the external solution. The second slow phase of absorption is due to additional passive absorption across the membranes into symplasm.

Effect of VAM fungi on atrazine uptake. The uninoculated (non-VAM) roots were free of VAM fungal infection and VAM (inoculated) roots were highly infected (Table 2). Atrazine absorption was measured with corn roots after 6, 7, and 8 wk of growth and with soybean roots at 6 and 7 wk of growth. Roots infected with VAM consistently took up more atrazine than the non-VAM roots in all the different time course studies conducted with corn and soybean (Figures 1 and 2). Also, absorption of atrazine increased with plant age in VAM-infected roots of corn (Figure 1a). Herbicide uptake was very similar in non-VAM corn roots at different stages of growth (Figure 1b). Similarly, no effect of plant maturity was found in atrazine absorption by excised velvet leaf roots (Abutilon theophrasti Medic) (28). The difference in absorption between mycorrhizal and non-mycorrhizal roots was the greatest in roots from older com plants. For instance, the absorption of 7 μM atrazine at 1440 min by roots from 8-wk old corn plants was 3.4 times higher than that of the corresponding
nonmycorrhizal roots, while it was only 1.8 times higher at 6 wk. Even though VAM colonization of soybeans enhanced herbicide uptake, the influence of VAM fungi on root absorption was less when compared with VAM effects on corn roots.

In all the experiments, care was taken to use the tertiary root segments for the absorption assays, assuming that these roots form the most active part of the root system. This may have reduced the effects of root age in this study. Root diameter was measured with a dissecting microscope and calculated root volume for the sample size (100-cm root length). Root volume was greater for the VAM-infected roots than for non-VAM roots (Table 2). In VAM roots, the internal VAM infection was comparable in the different aged roots. VAM-infected roots had greater uptake of atrazine than non-VAM roots, even after accounting for the differences in root volume between VAM and non-VAM treatments (Table 3), excepting soybean at 7 wk of growth.

The uptake rate constants $k$ and $k_Q$, calculated by a modified first-order regression model, described the pattern of atrazine uptake at different times by corn and soybean roots (Tables 4 and 5). The nonlinear regression model provided an excellent fit to the data based on an evaluation of the model F statistic and residual sum of squares. In general, the initial uptake rate is described by the rate constant $k$ with uptake reaching a maximum initial value ($S_Q$). The $k$ values for VAM roots were similar or less than for non-VAM roots, however, $S_Q$ values were much higher for VAM roots than for non-VAM roots. The parameter $k_Q$ is the rate constant describing absorption at later times. Thus, the parameters $S_Q$ and $k_Q$ both contribute to maximum uptake. Estimates of $k_Q$ that were not significantly different from zero (P<0.05) indicate no additional uptake above $S_Q$ with time. The small $k_Q$ values suggest a slow and gradual increase in uptake above $S_Q$. The $k_Q$ values for the 7 and 8 wk-old non-VAM corn roots at the 7-μM concentrations were zero or ten-fold less than for VAM-colonized roots (Table 4). The absorption by VAM corn roots was much higher at 1440 min than after 360 min, suggesting a continuing trend of absorption. Whereas, in non-VAM corn roots the initial rapid uptake reached a plateau with time (Figure 1b). The pattern of absorption reaching a plateau for roots without the VAM fungus reflects absorption solely by diffusion reaching an equilibrium with the external solution. The difference in the parameters $S_Q$ and $k_Q$ was less apparent between VAM and non-VAM soybean roots at 7-μM concentration (Table 5) compared with corn roots corresponding to the smaller difference in uptake between VAM and non-VAM soybean roots. In contrast to non-VAM roots, the increasing rate of atrazine absorption with time
and greater tissue accumulation by VAM roots suggests a VAM-fungus mediated uptake rather than a simple diffusion reported for atrazine absorption.

At the 0.7-μM concentration, herbicide absorption by both VAM and non-VAM roots reached a plateau quickly, suggesting an equilibrium with the external solution. (Figure 1 and 2). However the 7-wk old mycorrhizal soybean roots continued to take up herbicide over the entire time course. The $k_0$ values for 0.7 μM ranged from zero to a ten-fold less than $k_0$ values for absorption at 7 μM (Table 4 and 5). To our knowledge, none of the previous studies on herbicide absorption by excised roots included VAM colonized roots and hence a comparison on the efficiency of mycorrhizal roots with nonmycorrhizal roots for herbicide uptake is not available in the literature.

**Trifluralin absorption by soybean roots.** Excised VAM and non-VAM roots of soybean had the same pattern of trifluralin uptake as that of atrazine (Figure 3). This is consistent with the rapid uptake of trifluralin described in previous studies (3). The effect of VAM infection on root absorption of trifluralin was consistent with effect on atrazine uptake. The tissue concentration of trifluralin was higher than atrazine in all the situations. The movement of neutral, lipophilic xenobiotics across the lipid bilayer of the plasma membrane is mainly via non-facilitated diffusion, and the rate of absorption by plant cells is related to the lipophilicity of the compound (6, 30, 32). Membrane permeability controls solute entrance into the symplasm. Trifluralin is more lipophilic than atrazine as indicated by log $K_{OW}$ (octanol water partition coefficient) values of 5.5 for trifluralin (25) and 2.5 for atrazine (2). The higher lipophilicity of trifluralin accounts for the greater permeation of cell membranes and accumulation in tissues than with atrazine. Darmstadt et al. (6) found that the absorption of hydroxyatrazine was limited mainly to the apoplast where as the more lipophilic triazines, atrazine and ametryne, penetrated rapidly into the entire tissue volume of excised corn roots. Detailed studies on trifluralin uptake by different aged plants were not conducted.

**Herbicide Uptake by Intact Root Systems**

**Atrazine and trifluralin absorption.** Absorption of atrazine and trifluralin by intact root systems of soybean and corn, respectively, also showed greater uptake of herbicides by VAM roots than non-VAM roots (Table 6). However, the results showed a trend in atrazine uptake that differed from excised root study. The difference in atrazine uptake by VAM and non-VAM soybean roots was very low. But, trifluralin uptake by VAM colonized corn roots was 22 to 41% greater ($P<0.09$) than uptake by non-VAM roots. The mean dry weights of
soybean roots were 1.6, 3.9, and 2.7 g for non-VAM, non-VAM fertilized with P, and VAM roots, respectively. The corresponding corn root weights were 4.2, 11, and 9.1 g. Uptake by whole root systems was measured on a dry-weight basis, which includes larger, older roots that do not support an active VAM association. These older roots may have reduced the impact of VAM fungi in atrazine uptake by the whole root system. In both corn and soybean, P nutrition of plants did not significantly affect the herbicide uptake. The use of intact root systems without shoots enabled us to compare the efficiency of different root systems for herbicide uptake by eliminating any localized effect in excised root studies and in the absence of other environmental factors.

Herbicide Uptake by VAM External Hyphae

**Plant growth and VAM colonization.** Corn growth was greatly increased due to VAM infection. VAM-infected corn root systems were 895% larger than non-VAM plants. The mycorrhizal corn plants formed a root mat approximately 0.5-cm thick over the nylon screen, which constituted almost 50% of the total root weight and completely covered the screen. In non-VAM plants, root growth was reduced and matting over screen was negligible. VAM infection in inoculated plants was above 80%. Non-VAM treatments remained sterile with respect to VAM fungi. Live spore counts in soil from immediately below the nylon screen averaged 40 spores g⁻¹ soil in VAM-inoculated treatments. The high count of chlamydospores in soil below the screen confirmed VAM hyphal penetration and proliferation into the herbicide-treated soil. Direct microscopic examination also showed the hyphal network in soil.

**¹⁴C-atrazine uptake.** The VAM hyphal uptake of atrazine was evident by the presence of ¹⁴C-herbicide in VAM inoculated corn plants (Table 7). However some ¹⁴C was present in the non-VAM plants, which suggests that herbicide movement across the screen occurred. The concentration and total amount of ¹⁴C-atrazine residues in VAM plants were significantly (P<0.0003) higher than in non-VAM plants. The concentration of ¹⁴C in VAM plants after accounting for the ¹⁴C in nonmycorrhizal plants shows VAM hyphal uptake and transfer to corn plants. The ¹⁴C present in the upper chamber may have resulted from migration of the herbicide or metabolites across the screen through volatilization and or through water movement, although care was taken to prevent excessive watering.

The plant accumulation of atrazine residues by *G. epigaeus* hyphal uptake was small when compared with the uptake by *G. epigaeus*-colonized and non-VAM corn grown in atrazine-treated soil where the plant accumulation varied from 10 to 202 µg g⁻¹ tissue
Nelson and Khan (22) also showed that hyphae-alone uptake of atrazine was very low compared with the uptake by VAM and non-VAM root systems. In both situations the experimental systems were similar and only a small portion of the total hyphal system may have penetrated through the screen into the herbicide-treated soil.

In these experiments, root systems colonized with VAM fungus consistently exhibited greater potential for herbicide uptake than non-VAM roots. All the evidence indicates that colonization by VAM fungus enhanced the efficiency of herbicide uptake by roots and established direct uptake by the fungal hyphae as one possible mechanism. The external hyphal length was not quantified, but an increased amount of extramatrical hyphae was very evident in older mycorrhizal roots in microscopic examinations. The external hyphae of *Glomus mosseae* (Nicol. & Gerd.) associated with corn plants was measured as 4 to 5 m g⁻¹ soil within the zone <5 mm from the root surface (14). The external hyphae can be very extensive as reported from subterranean clover (*Trifolium subterraneum* L.). *Glomus sp.* produced 2 to 14-m hyphae cm⁻¹ infected root length and it varied greatly between VAM species (1). External hyphae increased from nondetectable levels at 4 wk of growth to 14 m cm⁻¹ root after 6 wk of growth. Similar hyphal growth was expected in the present study also.

The enhanced atrazine uptake by VAM vs. non-VAM roots and increased uptake by VAM roots from older plants appears to be related to the greater volume and surface area of extramatrical hyphae. Increased absorption of mineral ions by VAM fungi is due to increased surface area contributed by the fungal hyphae and greater exploitation of soil or due to greater ion affinity of the fungal absorption sites or a combination of both factors (4, 16, 12, 31). The VAM association with plant roots has been found to alter the root morphology (14) and root properties such as hydraulic conductivity (10, 23) and membrane permeability (8), which may also affect the root uptake mechanisms. Therefore, the use of VAM-colonized excised roots did not provide conclusive evidence of increased root uptake due to hyphal transfer of herbicides to root tissues. The direct VAM hyphal uptake of atrazine suggests that hyphal mediated uptake also contribute for the increase in herbicide uptake by mycorrhizal roots. Evidence for VAM hyphal mediated uptake of pesticide residues from soil was also presented in previous studies (21, 22).

These findings indicate that VAM may increase herbicide uptake in field situations. However, under field conditions, the herbicide availability may be limited by several factors not examined in this research, and therefore projection of these results to field situations is not yet possible.
LITERATURE CITED


Figure 1. Time course of $^{14}$C- atrazine absorption by excised roots from 6, 7, and 8 wk old corn plants. Solid lines indicate regression lines generated using non-linear regression models.
Figure 2. Time course of $^{14}$C-atrazine absorption by excised roots from 6 and 7 wk old soybean plants. Solid lines indicate regression lines generated using non-linear regression models.
Figure 3. Time course of $^{14}$C-trifluralin absorption by excised soybean roots. Solid lines indicate regression lines generated using non-linear regression models.
Table 1. Absorption of 7 μM atrazine by excised roots of different aged corn and soybean plants over a 1440 min time course\(^a\).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Absorption</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VAM</td>
<td>non-VAM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 wk</td>
<td>7 wk</td>
<td>8 wk</td>
<td>6 wk</td>
<td>7 wk</td>
<td>8 wk</td>
</tr>
<tr>
<td>Corn</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>12</td>
<td>37</td>
<td>54</td>
<td>79</td>
<td>63</td>
<td>66</td>
</tr>
<tr>
<td>120</td>
<td>51</td>
<td>40</td>
<td>65</td>
<td>82</td>
<td>83</td>
<td>89</td>
</tr>
<tr>
<td>240</td>
<td>77</td>
<td>56</td>
<td>69</td>
<td>91</td>
<td>69</td>
<td>93</td>
</tr>
<tr>
<td>360</td>
<td>84</td>
<td>62</td>
<td>69</td>
<td>98</td>
<td>90</td>
<td>91</td>
</tr>
<tr>
<td>1440</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Soybean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>51</td>
<td>—</td>
<td>71</td>
<td>60</td>
<td>—</td>
</tr>
<tr>
<td>120</td>
<td>70</td>
<td>65</td>
<td>—</td>
<td>91</td>
<td>76</td>
<td>—</td>
</tr>
<tr>
<td>240</td>
<td>83</td>
<td>65</td>
<td>—</td>
<td>88</td>
<td>90</td>
<td>—</td>
</tr>
<tr>
<td>360</td>
<td>70</td>
<td>63</td>
<td>—</td>
<td>91</td>
<td>80</td>
<td>—</td>
</tr>
<tr>
<td>1440</td>
<td>100</td>
<td>100</td>
<td>—</td>
<td>100</td>
<td>100</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\text{Dashed lines indicate that absorption study was not conducted at 8 wk growth.}\)
Table 2. Root volume and VAM infection of different aged corn and soybean roots used in atrazine uptake experiments.a.

<table>
<thead>
<tr>
<th>VAM fungus</th>
<th>Root Volume&lt;sup&gt;b&lt;/sup&gt; (mm&lt;sup&gt;3&lt;/sup&gt; (100 cm root)&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>VAM Infection&lt;sup&gt;c&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 wk</td>
<td>7 wk</td>
</tr>
<tr>
<td>Corn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAM</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>non-VAM</td>
<td>29</td>
<td>28</td>
</tr>
<tr>
<td>Soybean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAM</td>
<td>62</td>
<td>64</td>
</tr>
<tr>
<td>non-VAM</td>
<td>53</td>
<td>52</td>
</tr>
</tbody>
</table>

<sup>a</sup>Dashed lines indicate that measurements were not made at 8 wk growth.

<sup>b</sup>Volume calculated from mean diameter (average of 10 observations).

<sup>c</sup>Mean value of 4 observations followed by standard deviations.
Table 3. Atrazine absorption at 1440 min by different aged corn and soybean roots expressed on root volume basis.

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>VAM</th>
<th>non-VAM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 wk</td>
<td>7 wk</td>
</tr>
<tr>
<td>Corn:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>7</td>
<td>17.4</td>
<td>17.3</td>
</tr>
<tr>
<td>Bean:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>1.76</td>
<td>4.5</td>
</tr>
<tr>
<td>7</td>
<td>15.9</td>
<td>22.8</td>
</tr>
</tbody>
</table>

^Dashed lines indicate that measurements were not made at 8 wk growth.
Table 4. Kinetic parameters describing atrazine absorption by different aged excised roots of corn over a 1440 min time course.

<table>
<thead>
<tr>
<th>Growth (wk)</th>
<th>VAM</th>
<th>non-VAM</th>
<th>Uptake parameters&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k</td>
<td>$S_0$</td>
<td>$k_0$ b</td>
</tr>
<tr>
<td></td>
<td>h&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>pM</td>
<td>pM h&lt;sup&gt;-1&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.7 µM:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.009</td>
<td>0.65</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(0.003)</td>
<td>(0.09)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>7</td>
<td>0.03</td>
<td>0.46</td>
<td>$1.9\times10^{-4}$</td>
</tr>
<tr>
<td></td>
<td>(0.004)</td>
<td>(0.02)</td>
<td>($1.9\times10^{-5}$)</td>
</tr>
<tr>
<td>8</td>
<td>0.04</td>
<td>0.84</td>
<td>$4.4\times10^{-5}$</td>
</tr>
<tr>
<td></td>
<td>(0.001)</td>
<td>(0.006)</td>
<td>($7.2\times10^{-5}$)</td>
</tr>
<tr>
<td>7 µM:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.06</td>
<td>5.87</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(0.001)</td>
<td>(0.70)</td>
<td>(0.00)</td>
</tr>
<tr>
<td>7</td>
<td>0.06</td>
<td>3.11</td>
<td>$3.0\times10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>($4.1\times10^{-4}$)</td>
</tr>
<tr>
<td>8</td>
<td>0.07</td>
<td>6.76</td>
<td>$3.0\times10^{-3}$</td>
</tr>
<tr>
<td></td>
<td>(0.007)</td>
<td>(0.12)</td>
<td>($1.8\times10^{-4}$)</td>
</tr>
</tbody>
</table>

<sup>a</sup>$S_0$ is the maximum uptake, $k$ is the uptake rate constant for the initial absorption process (initial rate), and $k_0$ is the rate constant for the maximum uptake describing absorption at the later part of the time course obtained by non-linear regression analysis. Values in parenthesis are standard deviations.

<sup>b</sup>A value of zero indicates that estimates of $k_0$ were not significantly different from zero at $P\leq0.05$. 
Table 5. Kinetic parameters describing atrazine absorption by different aged excised roots of soybean over a 1440 min time course.

<table>
<thead>
<tr>
<th>Growth (wk)</th>
<th>Uptake parameters&lt;sup&gt;a&lt;/sup&gt;</th>
<th>VAM</th>
<th>non-VAM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k$ (h&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>$S_0$ (pM)</td>
<td>$k_0$ (pM h&lt;sup&gt;-1&lt;/sup&gt;)</td>
</tr>
<tr>
<td>0.7 μM:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.06 (0.02)</td>
<td>0.96 (0.07)</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0.08 (0.02)</td>
<td>0.68 (0.06)</td>
<td>7.5×10&lt;sup&gt;-5&lt;/sup&gt; (1.5×10&lt;sup&gt;-3&lt;/sup&gt;)</td>
</tr>
<tr>
<td>7 μM:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.05 (0.03)</td>
<td>6.91 (0.54)</td>
<td>1.9×10&lt;sup&gt;-3&lt;/sup&gt; (7.2×10&lt;sup&gt;-4&lt;/sup&gt;)</td>
</tr>
<tr>
<td>7</td>
<td>0.06 (0.02)</td>
<td>8.13 (0.38)</td>
<td>4.1×10&lt;sup&gt;-3&lt;/sup&gt; (5.0×10&lt;sup&gt;-4&lt;/sup&gt;)</td>
</tr>
</tbody>
</table>

<sup>a</sup>$S_0$ is the maximum uptake, $k$ is the uptake rate constant for the initial absorption process (initial rate), and $k_0$ is the rate constant for the maximum uptake describing absorption at the later part of the time course obtained by non-linear regression analysis. Values in parenthesis are standard deviations.

<sup>b</sup>A value of zero indicates that estimates of $k_0$ were not significantly different from zero at P ≤0.05.
Table 6. Absorption of atrazine by intact soybean root systems and trifluralin by corn root systems.

<table>
<thead>
<tr>
<th>VAM fungus</th>
<th>Herbicide absorptiona</th>
<th>VAM infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trifluralin by corn</td>
<td>Atrazine by</td>
</tr>
<tr>
<td></td>
<td></td>
<td>soybean</td>
</tr>
<tr>
<td></td>
<td>μM g⁻¹</td>
<td>%</td>
</tr>
<tr>
<td>VAM</td>
<td>0.73 ± 0.19a</td>
<td>0.07 ± 0.02a</td>
</tr>
<tr>
<td>Non-VAM</td>
<td>0.43 ± 0.13b</td>
<td>0.06 ± 0.02a</td>
</tr>
<tr>
<td>Non-VAM, + Pb</td>
<td>0.56 ± 0.05b</td>
<td>0.05 ± 0.004a</td>
</tr>
</tbody>
</table>

aMean value of three observations with standard deviation followed by the same letter within a column are not significantly different (P<0.05) as determined by the LSD test.

bNonmycorrhizal plants grown in low-P soil fertilized with 60 mg P kg⁻¹ soil.
Table 7. Amounts of $^{14}$C-atrazine residues in root and shoot of corn.

<table>
<thead>
<tr>
<th>VAM fungus</th>
<th>Root</th>
<th>Shoot</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAM</td>
<td>40.0±20.8a</td>
<td>4.3±0.6a</td>
<td>4.3±0.6a</td>
</tr>
<tr>
<td>Non-VAM</td>
<td>8.1±10b</td>
<td>0.8±0.7b</td>
<td>0.2±0.02b</td>
</tr>
</tbody>
</table>

Note: Amounts determined by oxidation and trapping of $^{14}$C and given as mean value of four observations with standard deviation. The different letters after the values within a column show that means are significantly different (P<0.05) as determined by the LSD test.
IMPACT OF CORN AND A VA MYCORRHIZAL FUNGUS (GLOMUS EPIGAEUS) ON DEGRADATION AND UPTAKE OF ATRAZINE IN SOIL

A paper to be submitted to Journal of Environmental Quality
Mercy J. Nedumpara, Thomas B. Moorman, and Elizabeth A. Douglass

ABSTRACT

The role of vesicular arbuscular mycorrhizal (VAM) fungi on the degradation of pesticides in plant rhizosphere is not known. The effect of corn (Zea mays L.) and the symbiotic VAM fungus, Glomus epigaeus (Daniels & Trappe), on degradation and plant uptake of atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) in soil was investigated. Soil was sterilized to eliminate indigenous VAM fungi. Atrazine-treated soil was inoculated with a heterotrophic microbial population other than VAM fungi for 10 d before planting with corn. Corn plants were grown in soil treated with $^{14}$C-atrazine containing G. epigaeus inoculum or without the VAM fungus. Treatments also included soil without any plants. Plants were harvested at 5, 8, and 11 wk after planting. By 45 d after atrazine application the concentration of atrazine present in different soils was <10% of the initial concentration applied. This result indicates that atrazine breakdown in soil was not affected by corn or G. epigaeus. At 11 wk after planting 27, 16, and 23% of the total $^{14}$C applied remained as the bound, unextractable fraction in VAM-corn soil, non-VAM corn soil, and non-planted soil, respectively. Bound residues in the rhizosphere zone accounted for only 1 to 2% of applied $^{14}$C. Uptake by VAM and non-VAM corn amounted to almost 10% of the total $^{14}$C applied to soil by 11 wk after planting and the tissue concentrations were 40 and 18 µg g$^{-1}$, respectively. Mycorrhizal fungi increased the efficiency of plant uptake of atrazine, but plant size was the principle factor controlling total uptake during the early stages of growth. Plant removal together with binding immobilized greater amount of $^{14}$C in the corn systems than in non-planted soil. The total loss of $^{14}$C from different soils exceeded 50% of the total $^{14}$C-atrazine applied by 45 d after atrazine application, presumably by volatilization and mineralization.
INTRODUCTION

Persistence and migration of pesticides are two major concerns in pesticide management. Plant rhizospheres constitute a large portion of the top soil and rhizosphere-biological activity influenced by plant is a significant aspect of this ecosystem. For instance, the perennial grass, crested wheat (Agropyron cristatum L.) produces up to 200,000 m roots m⁻³ soil which constitute a large surface area (Shimp et al., 1993). Plants enrich the root zone with various organic substances by rhizodeposition and root exudation (Barber and Martin, 1976; Krafczyk et al., 1984). Thus rhizosphere provides a nutrient rich microhabitat for microorganisms and increased biomass has been often observed in the rhizosphere (Rovira, 1973; Cheng and Coleman, 1990). Decreased persistence of xenobiotics, including pesticides, has been observed in planted soils compared to nonplanted soils (Hsu and Bartha, 1979; Reddy and Sethunathan, 1983; Ferro et al., 1994). In some instances larger degrader populations were observed in pesticide-treated rhizosphere soil than in control soils (Sandman and Loos, 1984). The greater enzyme activity in the rhizosphere by root-associated microbes and roots may enhance degradation of pesticides in rhizosphere soil. Plants may also play an important direct role in removal of pollutants by absorption and accumulation of organic compounds in their vegetative parts (Shimp et al., 1993).

Atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is a triazine herbicide widely used for the selective control of broad leaf and grassy weeds in corn. Degradation of atrazine in soil occurs by both biotic and abiotic processes (Skipper and Volk, 1972; Winkelman and Klaine, 1991). Most of the studies on biodegradation of atrazine in soil considered only microbial degradation. However, in agricultural soils, a knowledge of the effect of plants including plant-microbe-pesticide interactions is needed to fully address the biotic processes in the fate of field-applied chemicals. Root uptake, rhizosphere degradation and bound-residue formation are the major processes that are influenced by plants on the fate of chemicals in a cropped soil. These effects by plant depend mainly on the physical (root surface area) and chemical (exudation) characteristics of the root system.

Colonization of plant roots by VA-mycorrhizal fungi change the size and composition of the microbial population in the rhizosphere (Ames et al., 1984; Meyer and Lindeman, 1986; Secilia and Bagyaraj, 1987), presumably due to an effect on plant exudation (Dixon et al., 1989; Paulitz and Lindeman, 1991). Differences observed
between VAM and non-VAM rhizosphere microflora were often more on the size and composition of specific taxonomic or functional groups of bacteria more than a change in the total population. The change in microbial population and activity may influence the utilization and degradation of different organic compounds in the rhizosphere. The presence of VAM extramatrical hyphal network increase the effective root surface area (Jakobsen et al., 1992). Enhanced herbicide uptake by VAM-colonized roots and the direct role of VAM fungal hyphae on herbicide uptake have been observed in previous studies (Nelson and Khan, 1992; Nedumpara et al., 1996a). However, to our knowledge no information is available on the effect of VAM fungi on pesticide degradation in the rhizosphere. The role of VAM fungi on pesticide degradation is hypothesized to involve the direct hyphal-mediated metabolism of the compound or selection of a degrader population due to the chemical changes in the rhizosphere by VAM colonization.

In this research we investigated the persistence of atrazine applied at field recommended rates in soil planted with corn colonized with VAM fungus, *Glomus epigaeus* or without the fungus. The specific experimental objectives of this study were to (1) compare the degradation and immobilization of atrazine in soil planted with corn and without any plant, and (2) assess the role of VAM fungus, *G. epigaeus* on degradation, binding, and plant removal of atrazine from soil.

**MATERIALS AND METHODS**

**Soil Preparation**

Sandy loam soil containing <15 mg kg\(^{-1}\) available P was collected from the long term corn fertility plots at the old Agronomy Farm of Iowa State University. Soil was sieved through a 4 mm mesh sieve to remove large aggregates and gravel. A sterile 3:1 soil and sand mixture was used as the growth medium in this study. The soil-sand mixture was sterilized by autoclaving twice at 121°C with a 24-h interval between autoclavings. The soil-sand mixture contained 6 mg kg\(^{-1}\) P and 1.7% organic matter with a pH of 7.6.

Soils were prepared with 3 different treatments consisting of: (1) autoclaved soil inoculated with the VAM fungus *G. epigaeus* at 500 spores kg\(^{-1}\) soil, (2) sterile soil with added 30 mg soluble phosphate (KH\(_2\)PO\(_4\) solution) kg\(^{-1}\) soil to obtain plant growth comparable to VAM-infected plants, and (3) sterile soil without plants. Atrazine, 99% purity (Chemservice, West Chester, PA) and [U-ring \(^{14}\)C]-atrazine (Sigma Chemical Co., St.
Louis, MO) with radiochemical purity of >98% were used to prepare the treatment solutions in methanol. The atrazine stock solution was prepared in methanol. Soils (3 kg on oven-dry (O. D) basis) were treated with the [U-ring $^{14}$C]-atrazine solution to obtain a concentration of 2 mg kg$^{-1}$ soil on O. D basis. The specific activity of the treatment solution was 0.02 μCi mg$^{-1}$. Herbicide-treated soil was inoculated with soil (6 to 7 g) containing vegetative hyphae and chlamydospores of G. epigaeus from asparagus (Asparagus officinalis L.) pot culture, to get the inoculum density at 500 spores kg$^{-1}$ soil. The treated soil was then transferred to closed bottom, 18 cm, plastic pots. The herbicide-treated sterile soils were reinoculated with 100 ml of a soil suspension and incubated for 10 d before planting with corn seeds, to re-establish the heterogeneous populations of fungi and bacteria removed by autoclaving. The microbial inoculum was prepared by blending 100 g of the non-sterile soil with 1 L of water. Low P soil used for the preparation of growth medium was also used for this inoculum. The soil in the slurry was allowed to settle and the supernatant was passed through a 38-μm sieve to remove the VAM fungal inoculum. This filtered solution contained a mixed heterotrophic population which was not further characterized.

**Plant Growth**

Corn seeds (variety 8543, ICl, Slater, IA) were planted in soil treated with atrazine for treatments including plants. Pots were placed according to a completely randomized design in a growth chamber equipped with artificial (400-W high pressure Na lamps) lighting. Com was planted at two seeds per pot. Plants were then thinned to one per pot at the two-to-three leaf stage. Plants were fertilized with 50 ml of nutrient solution (Hoagland and Arnon, 1950) without P at weekly intervals and were grown under a 12 h photo period at 30°C. Four replicate plants were harvested at 5, 8, and 11 wk after planting.

**Plant Harvest**

The shoots and roots were harvested separately. The root along with the soil was removed from the pot and gently tapped to remove the bulk (nonrhizosphere) soil. Root samples were taken at random from the root system. The roots along with the adhering thin layer of rhizosphere soil was transferred to tubes containing 50 ml of 80% methanol -
water mixture (v/v). Root sampling resulted in approximately 20 g soil per tube. The tubes were shaken on a reciprocating shaker (Eberbach Corp., MI) at low speed for 30 min to dislodge the soil particles sticking to the roots. The roots were removed, dried at 60°C, and ground to determine the root absorption of atrazine. The rhizosphere soil in 80% methanol was extracted for atrazine. Additional rhizosphere samples were collected in a similar manner to determine the microbial populations. The roots and adhering soil were transferred to 90 ml of sterile phosphate buffer solution at pH 7.1 and shaken for 30 min prior to preparation of serial dilutions for plating. The bulk soil was mixed and subsamples were taken for estimation of microbial populations and atrazine concentration.

Chemical Analysis

Atrazine in soil samples were extracted using 50 ml of 80% methanol. Rhizosphere soil adhering to roots was collected in 80% methanol. Methanol extracts were shaken on a reciprocating shaker at low speed for 1 h. The samples were then centrifuged at 5000 X g for 15 min and the supernatant was collected. An additional 50 ml of 80% methanol was transferred to the soil, shaken for 1 h and allowed to sit for 24 h. The soil in methanol was shaken again for 1 h, centrifuged and the supernatant was collected. The supernatants were then pooled and diluted to 1 L with distilled water resulting in a 8% methanol-water mixture (v/v). This solution was shaken and 3-ml samples were taken and mixed with 15 ml of liquid scintillation fluid (Ultima Gold, Packard Instrument Company, Meriden, CT) and the radioactivity were measured using liquid scintillation spectrometry (1600 Packard Scintillation Counter, Packard Instrument Company, Meriden, CT). The total radioactivity in the 1 L solution was used to calculate the total extractable fraction of atrazine or metabolites in soil.

The diluted (1 L) methanol-water solutions were passed through a solid-phase extraction (SPE) cyclohexyl column (1000 mg, United Chemical Technologies, Inc. PA) by using a modified method described by Turin and Bowman, (1993). The SPE column was fixed on a rubber cork fitted with valved teflon needles with a one-way stopper and placed on a 1 L side-arm Erlenmeyer flask attached to a vacuum manifold (Alltech, IL). The valve was adjusted to regulate the flow of the solution through the column to approximately 2.5-to 3.5-ml hr⁻¹. The vacuum pressure was maintained between 3- to 4-mm Hg. The dilute methanol extract was drawn through the conditioned SPE columns using a teflon tube connected to the SPE column. After extraction, the column was allowed to dry for 10
to 15 min under vacuum. Atrazine sorbed to the SPE column was then eluted with 3 ml of methanol. Atrazine concentration in the methanol elute of the soil extract was determined by HPLC analysis. The HPLC system (Waters Corporation) consisted of an autosampler (model 712 WISP) and a programmable multiwavelength detector (Waters 490E UV). Liquid chromatographic separation of atrazine was achieved by an acetonitrile-water gradient (25:75) method using a Nova-Pak C18, 4 μM, 10 cm column employing Waters radial compression technology. Chemical concentrations were determined by using calibration curves constructed with atrazine standards (99% purity, Chemservice, West Chester, PA). Recovery from soils spiked with atrazine at 1 mg kg⁻¹ soil averaged 93% and data given for atrazine concentration are not corrected for the efficiency of extraction. The soil remaining after methanol extraction was allowed to dry and the unextractable fraction of atrazine and metabolites remaining in soil were determined by measurement of radioactivity in soil. The ¹⁴C content in soil was determined by combustion of a weighed amount of soil in a biological oxidizer (R. J. Harvey Instrument Co., NJ). The ¹⁴CO₂ evolved was collected in a special Harvee scintillation cocktail. The radioactivity trapped in the cocktail was measured by using liquid scintillation spectrophotometer with correction for quenching and background radioactivity. The efficiency of the oxidizer was >95%.

The shoot and root samples were dried at 60°C and dry weights were recorded. The samples were ground and the plant uptake of ¹⁴C-atrazine was quantified by measuring radioactivity in plant samples by the biological oxidation method as described above. From the ¹⁴C content and the initial specific activity of ¹⁴C-atrazine added to the soil, the concentration of atrazine was calculated using the assumption that ¹⁴C remained as the parent compound. The presence of metabolites will result in slightly under estimation of the concentration.

**Microbial Plate Counts**

Rhizosphere soil attached to roots was collected in buffer solution and was shaken on a reciprocating shaker at low speed for 30 min to dislodge the soil particles from roots. Subsamples from bulk soil and the rhizosphere soil collected in buffer solutions were used for the preparation of serial dilutions. Estimates of bacterial and fungal populations in rhizosphere and nonrhizosphere soils were made by the spreadplate method (Wollum, 1982). Fungi were enumerated on rose bengal agar medium and bacteria on 1% PTYG
(peptone-tryptone-yeast extract glucose agar). Fungal colonies were counted on day 3 and bacteria on day 7 after incubation at 21 to 23°C.

VAM Colonization

Subsamples of the fine tertiary roots were taken at random to determine VAM colonization. The roots were cleared and stained by the Philips and Hayman (1970) method. The total and VAM-infected root lengths were measured using the grid line-intersect method (Giovannetti and Mosse, 1980). The percentage of VAM root colonization was calculated from total and VAM-infected root lengths.

RESULTS AND DISCUSSION

Microbial Populations

Microbial populations in the rhizosphere and non-rhizosphere soil and the RS ratio (the ratio of the rhizosphere population to the non-rhizosphere soil population) are given in Table 1. The fungal and bacterial rhizosphere populations were larger than the bulk soil (non-rhizosphere) populations in both VAM and non-VAM corn soils as indicated by the RS ratio. In all cases bacterial populations were much larger than fungal populations. The temporal variations of bacteria and fungal populations were different in VAM and non-VAM rhizosphere. In the rhizosphere of mycorrhizal plants the fungal population decreased by 62% from week 5 to week 11 of plant growth while the bacterial population increased four fold (P<0.001) during the same period. Whereas, in the nonmycorrhizal rhizosphere, the fungal population increased by almost two orders of magnitude and the increase in bacterial population was almost two fold. In general, the total rhizosphere population increased with time. Earlier studies show that VAM fungi selectively influenced microbial populations of the rhizosphere and rhizoplane (Ames et al., 1984; Meyer and Linderman, 1986). Infection of blue grama (Bouteloua gracilis (H.B.K.) Griffiths) by Glomus mosseae (Nicol. and Gerd.) caused the proliferation of some gram negative bacterial groups in the rhizosphere while there was a reduction in the total population of bacteria (Ames et al., 1984).

The bacterial and fungal populations enumerated in nonrhizosphere soil of corn and non-planted soil are given in Table 1. The bacterial population in nonrhizosphere soil
increased with time. Whereas in non-planted soil fungal population increased and bacterial population decreased resulting in a decline in total population. The higher bacterial numbers in non-planted soil initially, when compared with nonrhizosphere soil of corn, could be attributable to the greater availability of mineral nutrients for microbes, in the absence of competition by plant roots allowing greater utilization of the available carbon sources. The population decline suggests a depletion of carbon and nutrients needed to sustain the population. The microbial population in planted soil exhibited a different trend from non-planted soil suggesting some interaction of plant and microbial population. The increase in bacterial population with the plant systems at 11 wk growth could be attributable to the increased supply of carbon released from the root and diffusion to the nonrhizosphere soil.

VAM Colonization

The VAM colonization of corn plants was low (Table 2) initially but by 8 wk of growth, the colonization was >70%. The slow establishment of the fungal symbiont caused the VAM plants to be smaller than the non-VAM plants, which were supplied with additional P (Table 2). However, the level of infection appeared to have less effect on herbicide uptake than P uptake by plants. Some other studies have noted that plant responsiveness to VAM fungi was not necessarily correlated with level of VAM infection (Clark and Mosse, 1981; Roldan-Fajardo, 1994). In another study, Nedumpara et al. (1996b) have seen that growth enhancement of soybean in low P soil by VAM species was not directly related to the extent of colonization suggesting that the effects of symbiosis by VAM fungi may not be strictly a function of the level of colonization.

Plant Uptake

The concentration of $^{14}$C-atrazine (calculated from $^{14}$C uptake assuming no transformation) in corn tissues varied from 6 to 202 μg g$^{-1}$ (Fig. 1). The concentration of $^{14}$C-herbicide in dry matter was significantly greater (P<0.0001) in VAM-colonized plants at the three different stages of corn growth. The shoot and root uptake of atrazine was 2.6 and 2.4 times, respectively, greater in VAM-infected plants on a per-gram-dry-mass basis when compared with non-VAM plants at 5 wk growth (Fig. 1). The corresponding increase in shoot and root concentration was 1.8 and 1.6 times at 11 wk of growth. There was
significant reduction in tissue concentration of $^{14}$C-atrazine in VAM and non-VAM corn plants with growth ($P<0.0001$). This trend was greater in VAM-infected corn plants. At 11 wk of growth the tissue concentration (shoot and root dry matter pooled together) of atrazine was eight and five times less than that at 5 wk in mycorrhizal and nonmycorrhizal corn, respectively. The reduction in concentration appears to be due to dilution caused by an increased plant mass with time. Metabolism and root discharge of small amounts of $^{14}$C to outside the plant was also a possible factor.

Although the herbicide concentrations were greater in VAM plants than in non-VAM plants, the total plant uptake of atrazine was greater by non-VAM than by VAM plants, due to the larger mass associated with non-VAM plants (Table 3). Non-VAM corn plants with the additional P supply were much larger than $G$. epigaeus colonized corn, particularly at the 5 wk sampling (Table 3). The differences in growth of VAM and non-VAM plants are probably due to the low P availability in the P unfertilized soil and the time required to form an effective symbiosis. At the end of the experiment the difference in plant mass was reduced, but the mass of VAM plants were still 55% of the non-VAM plants. At 11 wk of growth the total plant accumulation of herbicide by both VAM and non-VAM plants almost equaled and accounted to about 10% of the total herbicide applied to soil. The uptake of atrazine by plants from soil is a function of both solution concentration and mass flow in response to the amount of water transpired by plants (Walker, 1972), suggesting greater uptake of atrazine by larger plants. Although the VAM plants were smaller, their uptake of atrazine was similar to non-VAM plants at 11 wk of growth (Table 3) which suggests greater efficiency of herbicide uptake by mycorrhizal roots on a per unit mass basis. In another study by Nedumpara et al. (1996a), the authors observed that the ability of corn and soybean excised roots for $^{14}$C-atrazine uptake was greatly enhanced by $G$. epigaeus colonization. Direct uptake of atrazine residues by hyphal systems of $Glomus$ sp. and transfer to corn was also evidenced in studies by Nelson and Khan (1992) and Nedumpara et al. (1996a).

**Bound Residue**

The concentration of $^{14}$C nonextractable by methanol (bound fraction) expressed as atrazine (concentration calculated from $^{14}$C measured and specific activity, assuming that $^{14}$C remained as parent compound) in the rhizosphere soil varied from 0.47-0.90 µg g$^{-1}$ constituting 21 to 45% of the initial concentration (2 µg g$^{-1}$) applied to soil (Fig. 2a).
Similar to these results, up to 43% of $^{14}$C-atrazine applied was accounted as unextractable residues after 180 d of incubation in microcosms using non-sterile soil (Winkelmann and Klaine, 1991). The concentration of bound residue was greater in the rhizosphere of mycorrhizal corn plants ($P<0.001$) when compared with non-VAM corn plants. Since the rhizosphere microbial populations in both the corn systems were comparable (Table 1), the increased binding of atrazine in the mycorrhizal rhizosphere suggests a direct effect of VAM fungi. The VAM fungi were reported to alter the composition and concentration of root exudates in the rhizosphere (Dixon et al., 1989; Paulitz and Linderman, 1991). The HPLC chromatograms of methanol extracts of mycorrhizosphere soil showed a large number of peaks which were not present in non-VAM rhizosphere soil suggesting the presence of a number of organic compounds in the VAM rhizosphere. The network of fungal hyphae in the rhizosphere along with the increased concentration of organic compounds may have resulted in greater concentration of bound atrazine ($g^{-1}$ soil) in the mycorrhizal rhizosphere. The extensive growth of VAM hyphae was found to enhance aggregation of soil and organic fractions (Tisdall and Oades, 1979) and also in heavy-metal binding (Galli et al., 1994). However, non-VAM rhizospheres (on a per-plant basis) contained slightly larger amounts of $^{14}$C as bound residues than VAM due to the larger size of the root system of non-VAM plants (Table 3).

In both the VAM and non-VAM corn, the concentration of the bound fraction decreased with time in the rhizosphere ($P<0.001$), and the magnitude of reduction was much greater in the VAM rhizosphere. The methanol-extractable $^{14}$C in soil after 5 wk of growth accounted for <25% of the original concentration applied to soil (Table 3). The newly formed roots would be exposed to lower amounts of $^{14}$C. Thus further growth would have the effect of diluting the concentration of $^{14}$C in the rhizosphere. The decline could also be due to the release of the bound fraction in the rhizosphere soil. Previous reports show that the unextractable bound residues of pesticides can be released by microbial activity, and available for further degradation and absorption by plant (Racke and Lichtenstein, 1985; Kloskowski and Fuhr, 1987). Kloskowski and Fuhr (1987) showed that corn plants were able to absorb aged bound residues of [carbonyl-$^{14}$C] methabenzthiazuron (MBT) from soil. Nelson and Khan (1990) reported that *Glomus sp.* were able to remove and transfer bound residues of the insecticide fonofos (O-ethyl S-phenyl ethylphosphonodithioate) from soil to onion (*Allium cepa* L.) plants. In the present study also, the bound fraction of atrazine might have been partially available for further degradation and utilization by plant-microbe interactions which reduced its persistent in
rhizosphere soil. The presence of VAM hyphae in the rhizosphere showed a prominent effect on accelerating the bound residue formation of atrazine in the rhizosphere.

The fraction of nonextractable atrazine in nonrhizosphere soil ranged from 14 to 26% of the initial concentration of atrazine applied to soil (Fig. 2b). In bulk soil the order of binding of atrazine was VAM-corn soil>non-plant soil>non-VAM corn soil and were significant at P<0.005. In mycorrhizal and non-plant soils the amount of bound atrazine in bulk soil increased with time. The concentration of bound fraction almost remained unchanged in non-VAM corn soil. The size of the microbial populations in the three different systems were comparable and hence quantitative differences in the microflora were presumably not a factor in the reduced binding of atrazine in nonmycorrhizal corn bulk soil. The greater binding of atrazine in mycorrhizal soil suggests direct binding by fungal hyphae that may have extended into the soil for greater distances away from roots.

The concentration of bound atrazine was greater in the rhizosphere than in the bulk soil (Fig. 2). The difference between rhizosphere and bulk soil was very pronounced in the VAM treatments initially but almost equaled the bulk concentration at the end of the experiment. Formation of bound residues of pesticides in soil due to microbial activity has been previously reported (Katan and Lichtenstein, 1977; Bollag et al., 1980). Enhanced microbial activity in the rhizosphere as evidenced by RS ratios together with the presence of high concentrations of organic substances released by plants in rhizosphere may have contributed to the accelerated binding of atrazine in rhizosphere soil.

Atrazine Degradation

The degradation of atrazine in the different soils was very similar (Fig. 3). There were no sterile controls to determine abiotic degradation. In the non-VAM rhizosphere, only about 5% of the initial concentration was present at 5 and 11 wk of growth. The concentration of atrazine in VAM rhizosphere soil at 5 wk after planting amounted to 8% of the initial concentration and by 11 wk the concentration was reduced to 2.8% of the initial level. In a preliminary study conducted on effect of rhizosphere on degradation of atrazine, <10% of the initial concentration was present in both VAM and non-VAM corn rhizosphere soil 45 d after atrazine application (data not shown). The bulk soil concentrations (Fig. 3b) of atrazine at the different harvest periods were very close to the rhizosphere concentrations in soils planted with corn. Atrazine concentration in bulk soil was 8.1, 3.8, and 6.6% of the initial concentration in VAM-corn, non-VAM corn, and non-plant system,
respectively at 5 wk of plant growth. At 11 wk the corresponding soil concentrations were 1.6, 1.1, and 2.7% of the initial concentration.

Our results indicate that the breakdown of atrazine in soil was not affected by the presence of corn or G. epigaeus. Soil fungi, including ectomycorrhizal fungi (Donnelly et al., 1993; Mougin et al., 1994), and bacteria are capable of degrading and mineralizing atrazine (Mandelbaum et al., 1993, Radosevich et al., 1995). The lack of a significant difference in atrazine persistence between the different soils also indicates that the size or activities of the microbial populations capable of degrading atrazine were not affected by the presence of corn or G. epigaeus. Mineralization of atrazine was not accelerated in laboratory studies using rhizosphere soil (without live roots) from corn (Anderson and Coats, 1995). However, enhanced degradation of atrazine and a few other pesticides were observed in rhizosphere soil from different plants (Anderson et al., 1994; Boyle and Shan, 1994; Anderson and Coats, 1995).

Since we used a closed bottom pot in the study, there was no loss of atrazine by leaching, which allowed us to account for the loss of atrazine from the system (Table 3). The loss (unrecovered fraction) of 14C from each pot was calculated by subtracting the total fraction remaining in the system in plant and soil (including methanol extractable and non-extractable fractions) from the total quantity of 14C initially applied to each pot. The unrecovered fraction of 14C-applied was probably lost from the system by volatilization and mineralization. The total loss of atrazine from all the soil systems regardless of the presence of plant roots or G. epigaeus exceeded 50% of the total atrazine applied to the soil at 5 wk after planting (Table 3). Similar losses by volatilization and mineralization exceeding 50% were found in a field lysimeter study by Keller and Webber (1995).

Volatilization of atrazine was not measured in this experiment, but losses by volatilization is considered low (Whang et al., 1993). In our study, the temperature (30°C for 12 h), soil moisture content (approximately at field capacity), and air currents in the growth chamber were higher than what we generally observe under field conditions. These conditions may have caused increased volatilization of atrazine above the normal levels. However, we assume that the major loss of 14C from soil was due to mineralization of atrazine considering the high percentage of total loss from the system. The atrazine degrader population was not measured in this study and hence we are unable to correlate atrazine disappearance to the microbial population.

The amount of 14C lost from the VAM system initially was slightly less comparing the other two systems, as more amount of 14C remained in unavailable form mainly as
bound fraction in soil and some in plant. The data show that the net loss was fairly close between 5, 8, and 11 wk in different systems (Table 3). The results suggest that under present conditions of this study, the process during the first few weeks had profound significance on the fate of atrazine in soil and after that only low concentrations of atrazine were freely available in the soil for further binding or uptake.

CONCLUSIONS

This experiment was designed to describe how corn root systems along with associated microbial populations and VAM fungi influenced the degradation of atrazine applied to soil. The results of the present study did not show any evidence that corn or G. epigaeus, enhanced the rate of atrazine degradation. But, plant uptake accounted for up to 10% of the total 14C applied to soil. Significant binding of 14C-atrazine applied to soil occurred during the first few weeks after application, which reduced the availability of herbicide in soil for further transformation and movement. The rhizosphere concentration of bound residue was greater than nonrhizosphere soil, but the rhizosphere fraction contributed to only a small percentage of the total 14C applied to soil. Root colonization by G. epigaeus increased atrazine binding in soil when compared to nonmycorrhizal corn and non-planted soil and the efficiency of roots for herbicide uptake. Total loss of atrazine which we attribute to mineralization and volatilization, from different soils exceeded 50% of the initial dose in 45 d after application.

Our results suggest that greater uptake of atrazine by plants colonized with VAM fungi than by non-VAM plants could be expected in field situations when plant growth is comparable. Corn root systems and colonization by VAM fungi also contribute to the immobilization of herbicide by binding and the extent of bound residue formation is related to the volume of the root system. In summary, corn plants and G. epigaeus increase the uptake and binding of chemical in soil and consequently reduce the availability and potential leaching of herbicide in soil.

REFERENCES


Fig. 1. Plant uptake of $^{14}$C residues at different harvest times determined by oxidation and trapping of $^{14}$C.
Fig. 2. Bound $^{14}$C residues in soil at different harvest times determined by oxidation and trapping of $^{14}$C.
Fig. 3. Concentration of atrazine in soil at different harvest times determined by HPLC analysis.
Table 1. Populations of viable, aerobic, heterotrophic microorganisms in the corn rhizosphere and non-rhizosphere soil at different harvest periods.

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<tr>
<td>8 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAM</td>
<td>5.7x10³</td>
<td>1.4x10⁷</td>
<td>1.2x10³</td>
</tr>
<tr>
<td></td>
<td>(6.3x10³)</td>
<td>(2.2x10⁶)</td>
<td>(6.7x10²)</td>
</tr>
<tr>
<td>Non-VAM</td>
<td>8.4x10³</td>
<td>6.4x10⁷</td>
<td>4.8x10³</td>
</tr>
<tr>
<td></td>
<td>(5.6x10³)</td>
<td>(2.0x10⁷)</td>
<td>(4.5x10³)</td>
</tr>
<tr>
<td>Non-plant</td>
<td>—</td>
<td>—</td>
<td>2.4x10³</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1.8x10³)</td>
</tr>
<tr>
<td>11 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAM</td>
<td>1.7x10³</td>
<td>6.0x10⁷</td>
<td>1.2x10³</td>
</tr>
<tr>
<td></td>
<td>(1.0x10³)</td>
<td>(9.2x10⁶)</td>
<td>(4.5x10³)</td>
</tr>
<tr>
<td>Non-VAM</td>
<td>1.2x10⁴</td>
<td>5.0x10⁷</td>
<td>5.2x10³</td>
</tr>
<tr>
<td></td>
<td>(9.3x10³)</td>
<td>(1.6x10⁷)</td>
<td>(7.2x10³)</td>
</tr>
<tr>
<td>Non-plant</td>
<td>—</td>
<td>—</td>
<td>1.1x10⁴</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2.8x10³)</td>
</tr>
</tbody>
</table>

† Values given in parenthesis are standard deviations.
‡ Dashed lines indicate that no observations were recorded.
§ RS ratio is the ratio of rhizosphere population to non-rhizosphere population.
Table 2. Root colonization by *G. epigaeus* and dry matter of corn plants at different harvest periods†.

<table>
<thead>
<tr>
<th>Weeks</th>
<th>VAM colonization</th>
<th>Dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>G. epigaeus</em></td>
<td><em>G. epigaeus</em></td>
</tr>
<tr>
<td></td>
<td>Non-VAM</td>
<td>Non-VAM</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>g</td>
</tr>
<tr>
<td>5</td>
<td>35±9</td>
<td>&lt;1</td>
</tr>
<tr>
<td>8</td>
<td>75±4</td>
<td>&lt;1</td>
</tr>
<tr>
<td>11</td>
<td>77±6</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

† Mean value of 4 observations followed by standard deviations.
Table 3. Distribution and recovery of $^{14}$C at different stages of corn growth and G. *epigaeus* development†.

<table>
<thead>
<tr>
<th>Soil</th>
<th>Plant‡</th>
<th>Nonrhizosphere</th>
<th>Rhizosphere</th>
<th>MeOH bound§</th>
<th>MeOH bound§</th>
<th>Unrecovered extractable</th>
<th>(loss)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Total $^{14}$C</td>
</tr>
<tr>
<td>5 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAM</td>
<td>4.5±2.0</td>
<td>20.9±1.9</td>
<td>0.35±0.2</td>
<td>23.5±5.6</td>
<td>50.8±7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-VAM</td>
<td>8.5±1.4</td>
<td>16.2±3.3</td>
<td>1.1±0.6</td>
<td>12.6±7.1</td>
<td>61.6±5.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-plant</td>
<td>–</td>
<td>17.9±0.96</td>
<td>–</td>
<td>21.7±6.1</td>
<td>60.4±6.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAM</td>
<td>4.5±1.4</td>
<td>21.0±4.8</td>
<td>0.58±0.25</td>
<td>13.6±1.7</td>
<td>60.5±5.4</td>
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</tr>
<tr>
<td>Non-VAM</td>
<td>7.9±1.4</td>
<td>14.0±2.5</td>
<td>1.63±0.5</td>
<td>12.2±7.1</td>
<td>64.5±5.4</td>
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<td></td>
</tr>
<tr>
<td>Non-plant</td>
<td>–</td>
<td>22.8±3.3</td>
<td>–</td>
<td>12.4±4.9</td>
<td>64.9±2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAM</td>
<td>9.6±1.1</td>
<td>26.6±5.7</td>
<td>1.32±0.5</td>
<td>8.5±3.6</td>
<td>53.98±5.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-VAM</td>
<td>10.5±1.8</td>
<td>14.8±1.3</td>
<td>2.02±0.45</td>
<td>10.0±0.9</td>
<td>62.7±2.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-plant</td>
<td>–</td>
<td>22.8±2.2</td>
<td>–</td>
<td>13.4±4.2</td>
<td>63.8±8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>2.4</td>
<td>5.9</td>
<td>7.3</td>
<td>9.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Mean value of 4 observations followed by standard deviations.
‡ Dashed lines indicate that no observations were recorded.
§ Amounts determined by oxidation and trapping of $^{14}$C. Rhizosphere bound residues were calculated from soil/root weight ratios in samples and total root weight of plants.
GROWTH RESPONSE OF MYCORRHIZAL AND NONMYCORRHIZAL CORN AND SOYBEAN TO TRIFLURALIN AND ATRAZINE

A paper to be submitted to Agronomy Journal
Mercy J. Nedumpara, Thomas B. Moorman, and Krish Jayachandran

ABSTRACT

Carry-over of herbicide residues is a continuing field problem that interferes with the use of herbicide-sensitive rotational and replant crops. Corn (Zea mays L.) and soybean (Glycine max (L.) Merr.) are generally mycorrhizal and greenhouse experiments were conducted to study the effect of vesicular-arbuscular mycorrhizal (VAM) fungi on tolerance of these crops to herbicides. We examined the effect of a native population of VAM fungi and Glomus epigaeus (Daniels & Trappe) on the growth of soybean and corn in soils treated with atrazine (6-chloro-N-ethyl-N'-1-methylethyl)-1,3,5-triazine-2,4-diamine) and trifluralin (2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl) benzamine), respectively, at rates ranging from 0 to 1 mg kg⁻¹ in low P soil. Similar experiment was conducted in high P soil with atrazine on soybean. In low P soil, VAM fungi significantly enhanced P uptake and growth of soybean and corn compared to non-VAM plants at low doses of atrazine and trifluralin, which simulated field carry-over concentrations. In contrast to low P soil, VAM colonization of soybean was low in high P soil and there was no influence of VAM fungi on soybean growth or P uptake. The general trend in growth reduction due to herbicides was similar in VAM and non-VAM plants in both low and high P soils. Our results suggest that in low P soil the positive effect of VAM fungi on plant growth is more important than any negative effect due to VAM-herbicide interaction.

INTRODUCTION

The vesicular-arbuscular mycorrhizal (VAM) fungi are indigenous to most soils and form a symbiotic association within the roots of an enormously wide variety of host plants. Corn and soybean are two major agronomic crops and several VA mycorrhizal species have been found in association with these crops (Johnson et al., 1991; Khalil et al., 1992). Improved growth and yield due to increased concentrations of N, P, Ca, Mg, Zn and other micro nutrients have been reported for VAM infected soybeans and corn compared to
nonmycorrhizal plants (Ross and Harper, 1970; Ross, 1971; Lambert et al., 1979; Kothari et al., 1990; Khalil et al., 1994). Considerable variation is exhibited in the responsiveness or mycorrhizal dependency between varieties and cultivars of corn and soybean (Lambert et al., 1979; Khalil et al., 1994). However in P limiting situations, growth of most cultivars and varieties is positively correlated with VAM infection.

While beneficial effects of VAM fungi on nutrient uptake have been well documented, limited research has been done on the interactions of VAM fungi and pesticides. The significance of VAM fungi in plant response to herbicides is evident from three different types of interactions that are possible: (1) VAM fungi may increase plant uptake of herbicide in a similar manner to P uptake, which could result in crop injury, (2) VAM fungi may play a role in the degradation of herbicides or mediate increased detoxification by the plant, and (3) VAM fungi may play a role in protecting plants from herbicide injury by producing herbicide-safening compounds. Limited reports available suggest that VA-mycorrhizal association of plant roots may result in either increase or decrease in crop tolerance to herbicides. Increased herbicide phytotoxicity to VAM plants than to non-VAM plants has been observed by Busse and Ellis (1987) and Hammel et al. (1994). In contrast, VA-mycorrhizal plants showed reduced crop injury from herbicide application (Ocampo and Barea, 1985; Garcia-Romera and Ocampo, 1988; Siqueira et al., 1991). The contribution of VAM fungi to crop response may have significance when sensitive crops are exposed to herbicide residues.

Persistence and field carry-over of herbicide residues may cause problems to sensitive crops in rotational and replant situations, especially after long-term applications of the same herbicide. Atrazine and trifluralin are extensively used in Iowa. Atrazine was applied to nearly 61% of corn acres and trifluralin to 55% of soybean acres during 1990 in Iowa (Hartzler and Wintersteen, 1991). Field carry-over of both atrazine and trifluralin were seen in field studies and sometimes at levels that caused potential yield reduction (Oliver and Frans, 1968; Burnside et al., 1971; Marriage et al., 1975). Field studies conducted by Marriage et al. (1975) have shown that low concentrations of atrazine (0.4 kg ha\(^{-1}\)) present in soil from previous applications caused 38% yield reduction in oats (Avena sativa L.).

In our study, a series of experiments were conducted in the greenhouse to determine whether the interactions of VAM fungi and crops in soils treated with atrazine or trifluralin increased crop injury or crop tolerance. The specific experimental objectives were to (1) determine the effects of VAM fungi on the growth of soybean and corn in low and high P soils treated with atrazine and trifluralin, respectively, at rates simulating herbicide
carry-over and (2) assess the effect of these herbicides on VAM fungal colonization of roots. Moderate to high concentrations of available P are generally found in Iowa soils. A better understanding of the interactions between VAM fungi, crops, and herbicides may have implications in developing suitable herbicide-application strategies in protecting rotational and replant crops from herbicide injury.

MATERIALS AND METHODS

Soil Preparation and Growth Conditions

Soil was collected from the borders of long-term corn fertility plots at the old Agronomy Farm of Iowa State University, which were maintained at low available P status. The soil used in the experiments was a sandy loam containing 16% clay and pH 7.5. Chlamydospores of VAM fungi in the soil were estimated by wet sieving of the soil (Daniels and Skipper, 1982) using a nest of sieves (500 to 38 μm). The spores retained on the sieves were pooled together and systematically scanned under a stereo microscope at 40X magnification. Approximately 2 to 3 spores g⁻¹ soil, representing different species of VAM fungi, were present indicating an established native VA-mycorrhizal population. A 1:1 soil and sand mixture was used for the different studies. The soil and sand were sieved through a 4-mm mesh sieve to remove large aggregates and gravel before mixing. Soil-sand mixture contained 14- to 16-mg kg⁻¹ of available P (Bray 1) with a pH of 7.5. The mixture was sterilized by autoclaving twice at 121°C for 1 h, with 24-h duration between sterilization cycles.

For Glomus epigaeus treatments, soil inoculum consisted of chlamydospores and vegetative hyphae of the fungus collected from an asparagus (Asparagus officinalis L.) pot culture. The inoculum density (spore g⁻¹ soil) was determined by wet sieving method (Daniels and Skipper, 1982) and 5 to 6 g of pot culture soil resulted in an inoculum density of 500 spores kg⁻¹ soil. The VAM inoculum was uniformly mixed into herbicide-treated soil. Soil suspension containing heterotrophic bacterial and fungal population was added to sterile soils to re-establish the population removed by autoclaving. The heterotrophic microbial inoculum was prepared by blending 100-g low P field soil with 1 L of water. The slurry was allowed to settle and the supernatant was passed through 38-μm sieve to remove the VAM spores. The filtered solution (100 ml) was used as the inoculum.
The experiments were conducted in a completely randomized design in a greenhouse equipped with auxiliary lighting. The soybean variety Wells-II (Purdue University, IN) and corn cultivar 8543 (ICI Seeds Corp., Slater, IA) were planted at three seeds per pot after surface sterilization using 80% (v/v) ethanol for 1 min. Plants were thinned to one per pot at two to three leaf stage. Plants were watered daily to approximately field capacity. Care was taken to prevent leaching of herbicide due to excessive watering. The plants were fertilized with 50 ml of Hoagland's (Hoagland and Arnold, 1950) nutrient solution without P at weekly intervals. The plants were grown under a 14 h photo period at 27°C provided by 400-W high-pressure Na lamps.

**Herbicide Effects on Plant Growth Under Low P Conditions**

Experiments were designed to study the effect of VAM fungi on response of soybean and corn to herbicides atrazine and trifluralin respectively at doses that simulate concentrations resulting from herbicide carryover. Corn is tolerant to atrazine but susceptible to trifluralin. Soybean is (moderately) tolerant to trifluralin, but susceptible to atrazine.

Soils were prepared with three different VAM fungi and other heterotrophic soil microorganisms treatments consisting of: (1) the native population of VAM fungi and soil microorganisms, (2) autoclaved soil inoculated with the VAM fungus *G. epigaeus* at 500 spores kg⁻¹ soil and soil microorganisms, and (3) sterile soil inoculated with the heterotrophic soil microorganisms, but without any VAM fungi. Sterile soils were first treated with atrazine or trifluralin (both herbicides 99% purity, Chemservice, West Chester, PA) at rates 0, 0.05, 0.1, and 0.25 mg of herbicide kg⁻¹ soil on oven-dry (O. D.) basis. Atrazine and trifluralin stock solutions were prepared in methanol and 1 ml of these solutions were applied to 1 kg of sterile soil. The solvent was allowed to evaporate and the herbicide was mixed with the soil. In the preliminary study conducted using non-sterile field soil containing native VAM populations, herbicide concentrations 0.5 and 1 mg kg⁻¹ soil caused mortality or severe growth reduction and these doses were eliminated in the second study. Mycorrhizal inoculum was mixed into the herbicide-treated soil for *G. epigaeus* treatments. The herbicide-treated soil (3 kg) was transferred to 18-cm diameter plastic pots and 100 ml of soil suspension containing heterotrophic bacterial and fungal population was added to sterile soils. Soybean was planted in soil treated with atrazine
and corn was planted in trifluralin-treated soil. Each herbicide-plant-soil treatment included four replications. Plants were harvested 7 wk after planting.

Effect of Herbicides on VAM Fungi

The effect of trifluralin and atrazine on VA mycorrhizae formation were assessed by applying these herbicides on crops tolerant to the herbicides. This was done to eliminate the indirect effect on colonization due to reduction in plant growth by herbicide toxicity. VAM colonization of soybean and corn was measured after planting in low P soil treated with trifluralin and atrazine, respectively, at 0 and 1.0 mg kg\(^{-1}\) soil. Soil preparation and herbicide treatment were done as discussed previously. Two sets of experiments were conducted in greenhouse conditions using low P soil. In the preliminary study, the effect of these herbicides on colonization by native population of VAM fungi was measured. In the second set of experiments, the effect of herbicides on colonization by \textit{G. epigaeus} was measured. Heterotrophic microbial inoculum (100 ml) was added to the mycorrhizal and non-mycorrhizal sterile treatments in the second experiment. The mycorrhizal and microbial inocula were added to herbicide treated soil as described previously. Plants were harvested after 7 wk of growth in greenhouse conditions.

Herbicide Effects on Plant Growth Under High P Conditions

Mycorrhizal and nonmycorrhizal soybeans were grown in soil treated with atrazine at rates of 0, 0.05, 0.1, and 0.25 mg kg\(^{-1}\) in high P soil. The low P 1:1 soil and sand mixture was treated with 33 mg P kg\(^{-1}\) soil at planting. An additional dose of 33 mg P kg\(^{-1}\) was given 15 d after planting. A stock solution of KH\(_2\)PO\(_4\) was used as the source of P. The experimental design with respect to herbicides, VAM and microbial inoculum, application of treatments and growth conditions were the same as described for experiments with the low P soil. Plants were harvested 7 wk after planting.

Plant Harvest

The shoot and root systems were harvested separately and the fresh weights were determined. Plant materials were dried at 60°C for 2 d and weighed. The dried shoot and leaf portions were combined, ground, and digested in double acid (HNO\(_3\)/HClO\(_4\) at 1:1
ratio) at 160 to 200°C for 4 to 5 h. The P content of the digested samples was determined by the ascorbic acid method (Olsen and Sommers, 1982).

**VAM Colonization**

Fresh subsamples of the tertiary roots were taken at random to determine the VAM colonization. The roots were cleared and stained using the Philips and Hayman (1970) method. Trypan blue (0.025% strength) was used as the fungal stain. The VAM-infected root lengths and non-VAM root lengths were measured using the grid line-intersect method (Giovannetti and Mosse, 1980).

**Statistical Analysis**

Data were analyzed using ANOVA using SAS (SAS Institute Inc., Cary, NC) and LSD tests for mean comparisons. The herbicide dose-response relationship was estimated by a logistic dose response eqn $y=(a+b)/(1+(x/c)^d)$, where $c$ is the $I_{50}$ (dose giving 50% reduction in growth), $a$ is the lowest biomass of the treated plants, $b$ is the highest biomass of the treated or control plants, $x$ is the herbicide dose, $y$ is the plant growth, and $d$ is the slope of the curve. The analysis was done using TableCurve v3.0 software (Jandel Scientific Co., CA).

**RESULTS AND DISCUSSION**

**Effect of Herbicides on VAM Formation on Tolerant Crops**

The VAM fungus, *G. epigaeus*, significantly increased the growth of corn ($P<0.0001$) and soybean ($P<0.001$) plants compared to the nonmycorrhizal herbicide-treated and control plants (Table 1). *G. epigaeus* colonization of corn was reduced by 20% by atrazine at 1 mg kg$^{-1}$ ($P<0.03$). Trifluralin did not cause a significant reduction in VAM colonization of soybean. Similar results were obtained in a preliminary study on the effects of atrazine and trifluralin on colonization by the native species of VAM fungi (data not shown).

The herbicide effects on plant growth were small (Table 1), suggesting that a significant reduction in VAM colonization of roots relates to direct toxicity to VAM formation.
Atrazine is a photosynthetic inhibitor, but corn is able to rapidly metabolize atrazine (Shimabukuro 1967; Shimabukuro et al., 1971). Trifluralin affects plants by interrupting cell division and tolerance in soybean is expressed by tolerance to the biochemical effects of the herbicide. However, trifluralin has been shown to inhibit soybean root growth (Oliver and Frans, 1968). In other investigations, trifluralin did not exhibit direct toxicity to the infection and development of different VAM fungal species on various host plants (Burpee and Cole, 1978; Nemec and Tucker, 1983). Burpee and Cole (1978) observed that trifluralin applied at 4 kg ha⁻¹, much above the recommended rate, had no effect on colonization of soybean by native species. Atrazine did not affect the VAM symbiosis in Liquidambar styraciflua (Trappe et al., 1984). Some of other triazine herbicides like simazine and cyanazine also did not affect VAM formation at low to intermediate doses (Nemec and Tucker, 1983; Garcia-Romera et al., 1988). But simazine applied above field recommended rates, reduced VAM formation in Citrus spp. (Nemec and Tucker, 1983). In our study, a moderate toxicity by atrazine to colonization by native species and G. epigaeus was seen at field-recommended rates.

**Effect of Atrazine on Soybean Growth in Low P Soil**

Atrazine applied at 0.25 mg kg⁻¹ killed all the soybean plants. Soybean grown in atrazine-treated soil at rates of 0.05 and 0.1 mg kg⁻¹ were reduced in growth compared to the growth in untreated soil (Fig. 1). In the preliminary study we observed a similar pattern of growth reductions at lower atrazine concentrations and atrazine applied at 1 and 0.5 mg kg⁻¹ killed all soybean plants. The herbicide rates 0.5 and 1.0 mg kg⁻¹ represent concentrations that would be found immediately following applications in farmers fields and the lower concentrations represent concentrations found in field-carry over situations (Marriage et al., 1975). Soybean growth was significantly (P<0.0001) enhanced by both G. epigaeus and native population of VAM fungi than non-VAM plants at lower doses of atrazine application and in untreated soil. VAM-colonized and non-VAM soybean plants exhibited similar trends in growth reduction due to atrazine at lower doses, resulting in similar I₅₀ (dose giving 50% growth reduction) values (Table 2). Atrazine at 0.1 mg kg⁻¹ reduced plant dry mass by 46, 42, and 49% in nonmycorrhizal plants, plants colonized with native population and G. epigaeus, respectively. The pattern of shoot and root injury due to atrazine application was comparable in the different soil treatments and resulted in increase in shoot-root ratios (Table 2). The data show that effect of G. epigaeus and
native VAM species on soybean growth was comparable in atrazine treated and untreated soil. Garcia-Romera et al. (1988) reported that indigenous endophytes were equally effective as *Glomus mosseae* (Nicol. & Gerd.) in enhancing the growth of pea (*Pisum sativum* cv. Lincoln) plants in soil containing low concentration of cyanazine, another triazine herbicide.

The P content of VAM plants was significantly (P<0.0001) greater than that of non-VAM plants at all concentrations of atrazine, indicating that the VAM symbiosis was not affected by atrazine (Table 2). The shoot and leaf P concentrations of mycorrhizal soybean plants were almost two times greater than those of nonmycorrhizal plants in herbicide-treated and untreated soil. Atrazine reduced *G. epigaeus* root colonization by 18% but the colonization by the mixed population of VAM species was unaffected (Table 2), suggesting increased tolerance of the native population to atrazine. In the two sets of experiments, colonization by VAM fungi increased growth of soybean at 0.05 and 0.1 mg kg\(^{-1}\) concentrations of atrazine. Growth enhancement by VAM fungi was negated at higher concentrations of herbicide (≥0.25 mg kg\(^{-1}\)) and growth pattern matched to non-VAM treatments. Other investigators have reported that colonization by VAM fungi reduced crop injury at low to intermediate concentrations of herbicides when compared with nonmycorrhizal plants. The ability of different VAM species to reduce crop injury by herbicides has been demonstrated for chlorotoluron (N-[3-chloro-4-methylphenyl]-N,N-dimethylurea), MCPA (4-chloro-2-(methylphenoxy)acetic acid), cyanazine, and imazaquin (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid) (Ocampo and Barea, 1985; Garcia-Romera and Ocampo, 1988; Dodd and Jeffries, 1989; Siqueira et al., 1991). In most cases the alleviation of herbicide phytotoxicity was attributed to the growth promotion by VAM fungi due to increased P uptake.

**Soybean Growth in High P Soil**

The response of mycorrhizal and non-mycorrhizal soybean plants to atrazine application was similar in soils containing high concentrations of available P (Fig. 2). The total dry matter was not significantly different in mycorrhizal and nonmycorrhizal plants, which shows that there was no growth promotion of soybean plants by mycorrhizal fungi. The plant P content was not significantly different in the three different soil treatments (Table 3) indicating that there was no effect on P uptake by mycorrhizal fungi colonizing soybean. The reduction in plant dry mass due to low atrazine doses was comparable
between mycorrhizal and nonmycorrhizal plants. However, atrazine at 0.25 mg kg\(^{-1}\) caused greater damage to mycorrhizal plants and the dry mass of nonmycorrhizal plants was almost 2 times greater (P<0.10) than that of mycorrhizal plants (Fig. 2). The greater growth decline of VAM plants than non-VAM plants could be due to an increase in uptake of atrazine residues by mycorrhizal roots. Nedumpara et al. (1996) have observed that the ability of corn and soybean excised roots for \(^{14}\)C-atrazine uptake was greatly enhanced by \textit{G. epigaeus} colonization compared to non-VAM roots. The direct uptake of atrazine residues by hyphae of \textit{Glomus sp.} from soil and transfer to corn was reported by Nelson and Khan (1992) and Nedumpara et al. (1996). In studies where P is limiting, the increased P uptake by VAM fungi could have obscured the effect of the simultaneous uptake of herbicides. Busse and Ellis (1987) observed greater damage to VAM-infected soybeans from atrazine residues than to non-VAM soybeans in soil with a moderate P supply. Later, Hammel et al. (1994) reported that mycorrhizal colonization increased herbicide toxicity in apple. They observed increased mortality and phytotoxicity in \textit{Glomus versiforme} (Karsten) infected apple trees than in non-VAM apple after the application of herbicides simazine (2-chloro-4,6-bis-ethylamino-s-triazine), dichlobenil (2,6-dichlorobenzonitrile), and paraquat (1,1'-dimethyl-4,4'-bipyridinium).

The VAM root colonization was less than 15% (Table 3), compared to colonization exceeding 60% in soil containing low available P. Other researchers have also reported that mycorrhizae formation and development are inversely related to P supply (Bruce et al., 1994; Pearson et al., 1994). The lower VAM infection of soybean in high P soil probably reduced the network of external hyphae to a similar extent, which could have lessened the VAM mediated herbicide uptake and toxicity to plants.

**Effect of Trifluralin on Corn Growth in Low P Soil**

Corn growth was significantly (P<0.0001) improved by VAM fungi at all levels of trifluralin compared with non-VAM plants (Fig. 3). The \textit{G. epigaeus} was more effective than native species in increasing corn growth and root colonization (Table 4). Trifluralin at higher rates caused reduction in growth of corn. The decline in total dry matter was 22, 68, and 81% for corn plants colonized with native species, \textit{G. epigaeus} and non-VAM plants, respectively, at 0.25 mg kg\(^{-1}\) trifluralin. Corn plants colonized with native population of VAM fungi exhibited greater tolerance to trifluralin than \textit{G. epigaeus}-inoculated or nonmycorrhizal corn plants which results in the higher I\(_{50}\) values for plants colonized with
native population (Table 4). The shoot-root ratio of the plants with the native population also was relatively unaffected by trifluralin, while it increased greatly in the other two soil treatments (Table 4). High shoot-root ratio indicates reduced root development which is typical of trifluralin toxicity to com (Gentner and Burk, 1968). In the preliminary study conducted with native VAM species, similar trends were seen on VAM effect on corn growth and the pattern of growth reduction due to trifluralin in mycorrhizal and nonmycorrhizal corn (data not shown).

Trifluralin did not seem to have any adverse effect on mycorrhiza function as evidenced by the greater P content of VAM plants than non-VAM plants at different doses of the herbicide (Table 4). Corn shoot and leaf P content was significantly increased (P<0.05) by both the native mycorrhizal fungi and G. epigaeus, and the P content in plants from both VAM fungal treatments were comparable (Table 4). The similar tissue P concentrations, but difference in response to herbicide stress by mycorrhizal plants, indicate a VAM mediated tolerance (or safening) of corn to trifluralin by the mixed native species. The root infection of corn by G. epigaeus was reduced by 19% at 0.25 mg trifluralin kg⁻¹, whereas the native VAM species were unaffected (Table 4). The reduced G. epigaeus colonization of corn by trifluralin could be due to the greater herbicide stress in G. epigaeus-infected plants compared to plants colonized with the native population. Increased tolerance of the native population to trifluralin was also possible.

In general, soybean and corn growth was greatly improved by VAM fungi at lower concentrations of atrazine and trifluralin in low P soil. Growth improvement appears to be related to the enhanced P uptake. Soybean tolerance to atrazine was similar in soil containing G. epigaeus and the native VAM population in both low and high P soil. However, the tolerance of corn plants to trifluralin was greater in soil containing the native VAM population than G. epigaeus inoculum. The variation in VAM plants to herbicide tolerance could be attributed to the different VAM species in native population and the interaction among them. Siqueira et al. (1991) showed that native VAM species were able to alleviate the phytotoxic effect of imazaquin to sorghum, but the herbicide-safening effect was reduced when Glomus intraradix (Schenck and Smith) was added. Mycorrhizal fungal species, or even isolates of a given species, differ greatly in their effects on plants (Clark and Mosse, 1981; Roldan-Fajardo, 1994). The increased trifluralin tolerance of corn plants colonized by native population could be due to the differences in their efficiency for growth promotion or herbicide tolerances among VAM species.
In our study, the effects of atrazine and trifluralin on root infection by *G. epigaeus* and native population varied in the different experiments with low P soil. Differential tolerance of VAM fungal species to herbicide was observed in previous studies by Dodd and Jeffries (1989) and Garcia-Romera and Ocampo (1988). Garcia-Romera and Ocampo (1988) found that indigenous species were susceptible to MCPA at the rate of 10 mg kg\(^{-1}\) while a dose of 120 mg kg\(^{-1}\) was required to lower colonization of *G. mosseae*. The differential tolerance of VAM fungi to herbicide probably have influenced their effects on plant growth. However, the overall results of the study show that variations on the effects of different VAM treatments on plant growth are small considering the growth enhancement by VAM species when compared to non-VAM plants.

**CONCLUSIONS**

Trifluralin appears to have little direct effect on VAM formation as evidenced by the colonization of a tolerant crop, soybean. Whereas, the reduction in VAM colonization by atrazine on corn suggests moderate toxicity to the fungi. In low P soil the growth and P uptake of corn and soybean was significantly improved by VAM fungi at lower concentrations of herbicides when compared to nonmycorrhizal plants. The general trend in growth reduction due to herbicide was similar in mycorrhizal and non-mycorrhizal plants. In low P soil, the beneficial effect of VAM fungi on increased P uptake appears to be more important than any negative effect due to VAM-herbicide interaction. In contrast to low P soil, in high P soil the VAM colonization of soybean was low and the growth of mycorrhizal and non-mycorrhizal soybeans was similar at low doses. The growth of mycorrhizal soybean was less than nonmycorrhizal plants at 0.25 mg kg\(^{-1}\) of atrazine, suggesting injury due to increased uptake of herbicide by VAM roots. In high P fertility situations, due to the possible inhibition on VAM formation in plant roots, plant injury by VAM-mediated herbicide uptake may be limited.

**REFERENCES**


Fig. 1. Effect of atrazine on soybean growth at doses that simulate pesticide carry-over in low P soil. Hollow circles represent soil containing native population of VAM fungi, filled circles for sterile soil inoculated with *G. epigaeus*, and triangles for sterile soil without VAM fungi.
Fig. 2. Effect of atrazine on soybean growth at doses that simulate pesticide carry-over in high P soil. Filled circles represent sterile soil inoculated with *G. epigaeus*, hollow circles represent soil containing native population of VAM fungi, and triangles for sterile soil without VAM fungi.
Fig. 3. Effect of trifluralin on corn growth at doses that simulate pesticide carry-over in low P soil. Filled circles represent sterile soil inoculated with G. epigaeus, hollow circles represent soil containing native population of VAM fungi, and triangles for sterile soil without VAM fungi.
Table 1. The effect of herbicides on plant growth and colonization by VA-mycorrhizal fungi in a low P soil†.

<table>
<thead>
<tr>
<th>VAM fungi</th>
<th>Trifluralin on soybean</th>
<th>Atrazine on corn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbicide</td>
<td>dry matter</td>
<td>VAM</td>
</tr>
<tr>
<td></td>
<td>mg kg⁻¹</td>
<td>g</td>
</tr>
<tr>
<td>+ 0</td>
<td>4.7±0.7</td>
<td>88±9</td>
</tr>
<tr>
<td>+ 1</td>
<td>3.7±0.6</td>
<td>82±7</td>
</tr>
<tr>
<td>- 0</td>
<td>1.8±0.5</td>
<td>0</td>
</tr>
<tr>
<td>- 1</td>
<td>1.6±0.1</td>
<td>0</td>
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</table>

Analysis of variance (significance of F value)

<table>
<thead>
<tr>
<th></th>
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<th>&lt;0.001</th>
<th>&lt;0.0001</th>
<th>&lt;0.0001</th>
<th>&lt;0.0001</th>
</tr>
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<td>NS</td>
<td>NS</td>
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<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.03</td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
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<td>6.5</td>
<td>2.1</td>
<td>4.4</td>
<td></td>
</tr>
</tbody>
</table>

† Mean of four replicate observations and standard deviations
Table 2. The effect of atrazine on VAM colonization and P uptake of soybean in low P soil†.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Atrazine</th>
<th>VAM infection‡</th>
<th>Shoot-leaf P‡</th>
<th>Shoot-root ratio</th>
<th>I50§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg kg⁻¹</td>
<td>%</td>
<td>mg g⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native population</td>
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<td>0.25</td>
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<td>—</td>
<td>—</td>
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</tr>
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<td>72±6</td>
<td>1.5±0.04</td>
<td>3.34</td>
<td></td>
</tr>
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<td>0.25</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Non-VAM</td>
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</tr>
<tr>
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<td>0.25</td>
<td>—</td>
<td>—</td>
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</tr>
</tbody>
</table>

Analysis of variance (significance of F value)

- VAM: <0.0001
- Atrazine: <0.0001
- Interaction: <0.0001
- LSD (0.05): 6.43

† Dashed lines indicates plant mortality.
‡ Mean of four replicates and standard deviations.
§ Atrazine concentration giving 50% growth reduction.
Table 3. The effect of atrazine on VAM colonization and P uptake of soybean in high P soil.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Atrazine</th>
<th>VAM infection†</th>
<th>Shoot-leaf P†</th>
<th>Shoot-root ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native population</td>
<td>mg kg⁻¹</td>
<td>%</td>
<td>mg g⁻¹</td>
<td></td>
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<td>7±6</td>
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<td>G. epigaeus</td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<tr>
<td>non-VAM</td>
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<td></td>
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Analysis of variance (significance of F value)

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>VAM</td>
<td>&lt;0.001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Atrazine</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Interaction</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>6.61</td>
<td>0.9</td>
<td></td>
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</tbody>
</table>

† Mean of four replicate observations and standard deviations.
Table 4. The effect of trifluralin on VA mycorrhizal infection and P uptake of corn in low P soil.

<table>
<thead>
<tr>
<th>VAM fungi</th>
<th>Atrazine</th>
<th>VAM infection†</th>
<th>Shoot-leaf P†</th>
<th>Shoot-root ratio</th>
<th>l50‡</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>mg kg⁻¹</td>
<td>%</td>
<td>mg g⁻¹</td>
<td></td>
<td>mg kg⁻¹</td>
</tr>
<tr>
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<tr>
<td>Non-VAM</td>
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<tr>
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<tr>
<td></td>
<td>0.25</td>
<td>0.3±0.5</td>
<td>0.7±0.07</td>
<td>2.4</td>
<td></td>
</tr>
</tbody>
</table>

Analysis of variance (significance of F value)

VAM <0.0001   <0.001
Trifluralin <0.0001 <0.1
Interaction <0.01 <0.05
LSD (0.05) 8.3 0.2

† Mean of four replicates and standard deviations.
‡ Trifluralin concentration giving 50% growth reduction.
GENERAL SUMMARY

In this thesis I have attempted to examine the various facets in the interactions of VAM fungi, crops and herbicides. The overall objective of the study was to see whether VAM fungi contribute to the uptake of herbicides by plant root systems and degradation of herbicides in the rhizosphere and the consequent effect on herbicide tolerance by plants.

The results of the different herbicide uptake studies have demonstrated that the colonization of corn and soybean by VAM fungus, *G. epigaeus*, enhanced the efficiency of root uptake of atrazine and trifluralin. The difference in uptake between VAM and non-VAM roots increased with herbicide concentration, exposure time and age of the plant presumably due to the increase in external hyphal development. The mycorrhizal roots were larger than non-mycorrhizal roots, however mycorrhizal roots took up more herbicide even after accounting for the differences in root volume. Direct hyphal uptake of ¹⁴C-atrazine was evidenced in studies using a specially designed two chamber system in which only the *G. epigaeus* hyphae had access to the ¹⁴C-herbicide treated soil. In VAM roots, the presence of extensive network of external hyphae around the roots may have increased the effective surface area and volume per unit length and together with the hyphal mediated uptake may have contributed for the increased herbicide uptake by VAM roots.

Experiments conducted on effect of mycorrhizal and nonmycorrhizal rhizosphere on atrazine degradation and persistence in soil did not show any evidence that corn or *G. epigaeus* enhanced the rate of atrazine degradation in soil. Atrazine was degraded very rapidly in soil irrespective of the presence of corn or *G. epigaeus*. Total loss of atrazine from different soils exceeded 50% of the initial dose in 45 days after application presumably due to mineralization and volatilization. It was observed that binding of atrazine in soil during the first few weeks reduces its availability in soil and the rhizosphere and presence of *G. epigaeus* enhances bound residue formation. The results show that a profound rhizosphere contribution on binding relates to the rhizosphere volume of soil. The colonization of roots by *G. epigaeus* enhanced the efficiency of root uptake of herbicides.

Experiments were conducted in greenhouse conditions using low and high P soil that simulated exposure of soybean and corn to herbicides due to carry-over. Colonization by VAM fungi significantly enhanced P uptake and plant growth at lower concentrations of herbicides in low P soil. The VAM colonization of soybean in high P soil was very low and did not affect plant growth or P uptake. In general mycorrhizal plants were similar to
nonmycorrhizal plants in their response to herbicides in both low and high P soil. The results show that the positive effects of VAM fungi in promoting plant growth due to increased P uptake were more important than the negative effects due to increased herbicide uptake. However, greater damage to mycorrhizal soybean plants was found at higher rate of atrazine in high P soil although it was not a statistically significant effect. Plant damage from herbicides is controlled by a number of factors which may affect VAM-herbicide interactions that were not fully assessed in these experiments.

In several different kinds of experiments I have shown evidence that VAM fungi increase uptake of organic herbicides by plants. Increased uptake of herbicides by VAM fungi is likely to be of importance in situations where sensitive plants are exposed to herbicide residues. In agriculture, these situations arise due to carry-over of herbicide residues to the following replant or rotational crops. The finding of the study suggests that VA-mycorrhizae are only a minor agent affecting crop tolerance to herbicide residues. Another situation where enhanced plant uptake and degradation of herbicide residues is a consideration, is in the use of plants for on-site cleanup of pesticide residues. The greater efficiency of VAM plants for herbicide uptake and binding of herbicide in the soil suggest that VA-mycorrhizal plants could be effectively utilized in bioremediation approaches.
REFERENCES


