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THE ROLE OF VESICULAR-ARBUSCULAR MYCORRHIZA (VAM) IN HERBICIDE UPTAKE BY ROOTS OF WEEDS

BY

ALFRED J. KIPKORIR TARUS

A THESIS

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NAME OF AUTHOR Alfred J. Kipkorir Tarus

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(SIGNED) ---------

PERMANENT ADDRESS:

P. O. BOX 2997 NAKURU

KENYA.

DATED 6/12/93.

UNIVESITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled **The role of vesicular-arbuscular mycorrhiza (VAM) in herbicide uptake by roots of weeds** submitted by Alfred J. Kipkorir Tarus in partial fulfillment of the requirements for the degree of Master of Science in Weed Science.

Dr. W. H. Vanden Born (Supervisor)

Dr. J. P. Tewari

Dr. W. B. McGill

Date 17 NOV 1993

DEDICATION

To my father Kiptarus Kendagor and mother Teriki to whom I owe a lot for my success.

Abstract

Vesicular-arbuscular mycorrhiza (VAM) play an important role in the uptake of phosphorus and other nutrients by plant roots, particularly in soils that are low in these nutrients. The contribution of VAM fungi to the effectiveness of two soil-applied herbicides, atrazine and diclofop-methyl, on wild oats (*Avena fatua* L.) and green foxtail (*Setaria viridis* L.) was examined. In addition, the uptake of ^{14}C-labelled atrazine from soil by mycorrhizal hyphae associated with VAM-infected roots of green foxtail and white clover (*Trifolium repens* L.) plants was measured.

All three plants were infected readily by the mycorrhiza species used (*Glomus intraradices*). Fungal structures such as hyphae, arbuscules, and vesicles, and the extent of root colonization appeared unaffected by the presence of the herbicides.

There was no consistent evidence that VAM-infection contributed to more rapid or more extensive injury to wild oats or green foxtail plants growing in soil treated with atrazine or diclofop-methyl.

There was no significant difference in uptake of ^{14}C-atrazine (after 72 or 144 h) between VAM-infected and uninfected green foxtail and white clover plants. However, when the roots were confined to the upper compartment of specially constructed petri dishes so that only the hyphae made contact with the herbicide-treated soil in the lower

compartment, the hyphae supplied the plant with small amounts of the herbicide equivalent to 1-3% of that supplied by a complete root system. The VAM hyphae, therefore, can contribute to herbicide uptake by plants with infected roots, but their contribution is small.

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Introduction

Vesicular arbuscular mycorrhiza (VAM) fungi are cosmopolitan fungi that have been estimated to infect approximately 95% of all plant species including angiosperms and numerous gymnosperms, establishing symbiotic relationships (Freedman *et al.*, 1989; Trappe *et al.*, 1984; Garcia-Romera *et al.*, 1988). The most frequently cited benefits to the host plant are increased nutrient uptake and increased resistance to stress.

One of the major benefits of VAM association to the host is increased uptake of nutrients such as phosphorus, copper, zinc, and sulphur (Vivekanandan and Fixen, 1991). Hyphae of the fungi extend into the soil considerably beyond the root hairs, thus effectively extending the zone of nutrient absorption. The increase in uptake of the above elements improves plant growth, especially in soils that are deficient in these elements (Mosse, 1973).

There are reports that VAM fungi increase plant tolerance to drought stress (Allen and Boosalis, 1983). Hetrick *et al.* (1985), on the other hand, observed that mycorrhizal plants show no distinct improvement over non-mycorrhizal plants when grown under drought stress. There were increases in VA mycorrhizal root colonization but without corresponding benefits to the plant. It appears that VAM fungi may have a role in increasing water uptake of plants under certain conditions but the means by which this is achieved is unclear. There are also indications that VA mycorrhizal plants show greater tolerance to high temparatures than non-mycorrhizal plants (Grey, 1991).

There have been very few reports on the influence of VAM on the fate of pesticides in soils and plants. Busse and Ellis (1987) suggested that VAM inoculation could increase atrazine uptake from soil into soybeans, and Nelson and Khan (1992) recently reported that, in fact, VAM infection increased the uptake and transfer of ^{14}C-atrazine through a hyphal pathway to corn plants.

The objective of the research reported in this thesis was to examine the extent to which the presence of VAM contributes to the effectiveness of soil-applied herbicides on weeds, through a possible increase in herbicide uptake.

Two approaches were used to achieve this objective. First, the sensitivity of two VAM-infected weedy annual grasses to soil-applied herbicides was compared with that of uninfected plants. Secondly, ^{14}C-atrazine was used to quantify the contribution of VAM hyphae to uptake of this herbicide from the soil by plants.

For both experimental approaches, the herbicides used had to be effective via soil application, and the plants had to be both sensitive to the herbicides and susceptible to VAM infection. For the first series of experiments, the weed species wild oats (*Avena fatua* L.) and green foxtail (*Setaria virids* L.) were chosen as experimental materials because they are weeds of economic importance in Alberta crop land, and are susceptible to soil-applied herbicides and to VAM infection. Seed for these species was readily available and plants grew rapidly and uniformly. The herbicides atrazine and diclofop-methyl were chosen because they are effective via soil application, test plants are sensitive to them, and treated plants show growth inhibition and recognizable injury symptoms.

In the second series of experiments atrazine as the herbicide was selected because it had been shown by other researchers that VAM inoculation could increase its uptake from soil to crop plants and because its was available in a ^{14}C-labelled form. Green foxtail and white clover (*Trifolium repens* L.) were chosen as the test species because of their high susceptibility to VAM infection.

2. Literature Review

2. 1. Introduction

Vesicular-arbuscular (VA) mycorrhiza are soil fungi that form symbiotic associations with plant hosts. They have been identified as members of the *Glomaceae* family (Morton and Benny, 1990). In an infected plant, the mycorrhiza forms an integral part of the plant, and in nature most species (more than 95%) have a root system that is really a VA mycorrhizal system (Abbott and Robson, 1982; Smith and Gianinanzzi-Pearson, 1988).

Vesicular-arbuscular mycorrhizal fungi occur in almost all soils (Mosse *et al.*, 1981; Hetrick, 1984). The few instances where these fungi may be absent include eroded soils (where surface soil containing the fungi has been lost), fumigated soils (where fungi have been killed), or soils disturbed by mining (where either the removal of top soil or disturbance eliminates the fungi). The fungi are dispersed primarily by wind, soil movement, and animals (Abbot *et al.*, 1991). Because of this ability to disperse and because of their extensive host range, it is not surprising that VAM fungi are distributed widely.

Soils characteristically contain more than one species of VAM fungi. Many VAM species show wide ranges in their responses to edaphic and climatic factors. However, their identification is difficult, and many species of VAM fungi remain undescribed. At least 120 species of VAM fungi have been described (Morton, 1988) but relatively little is known about their global distribution.

Identification of species of VAM fungi in field soils is not easy without a substantial knowledge of these organisms. Descriptions

are based on the spore characteristics (Morton, 1988). The morphology of the fungus within roots also can be used, particularly where spores are not abundant or where the morphology of groups of fungi in a particular area has been studied extensively (Abbott, 1982). Recoginition of the VAM fungi (at least to the genus level) using characteristics of infection morphology requires a great deal of experience based on observations of mycorrhiza formed in pot cultures by a single species isolated from one area and studied on a single host plant. There may be some changes in the morphology of a given fungus within roots of different hosts (Abbot and Robson, 1982). Nevertheless, infection characteristics provide a valuable tool for understanding the relative extent of colonization of roots by species within a population (Abbot and Robson, 1982).

2. 2. Biology of VAM Fungi

It is important to understand the biology of the VAM fungi in order to predict their distribution and abundance in nature. Vesicular-arbuscular mycorrhiza are biotrophic in nature and colonization of roots is essential for their continued occurrence in soils (Harley and Smith, 1983). They have three different propagules: spores (in soil or within roots, depending on the species of fungus), hyphae (in soil or within living or dead roots), and vesicles (in roots).

Mycorrhizal roots on intact plants or germinated spores are understood as sources of infective hyphae for initiating new sites of colonization of roots. In addition, detached fragments of mycorrhizal roots can give rise to infective hyphae. Abbott and Robson (1981)

reported that the infectivity of some species of VAM fungi from this source depends on the stage of development of the infection within the root. Some species such as *Acaulospora laevis* which forms vesicles within roots were infective from relatively young infected root pieces but not from older root pieces. Vesicles formed within roots act as propagules for some fungi (Harley and Smith, 1983).

As well as understanding the biology of individual species of VAM fungi, we need to understand how species of VAM interact with one another during the formation of fungal stuctures such as spores, arbuscules, and vesicles. From glasshouse studies, it has been shown that the presence of one species of fungus can influence the quantity of fungal structures formed by another species (Lopes *et al.*, 1987). The most likely explanation of this effect is that the fungus with the more infective hyphae at the root surface is the one more likely to achieve substantial colonization (Abbott and Robson, 1984). Therefore, depending on the interaction of species present in a soil, the number of propagules of any one fungus may not determine the ultimate extent of mycorrhiza formation by that fungus. It has also been observed that the relative infectivity of propagules of different species is important. A fungus such as *A. laevis* may have a larger number of spores in the soil than another species but if those spores are dormant then other fungi without these limitations will form more fungal structures (Tommerup, 1983). In roots formed later, this pattern of infection may be reversed, as these new roots will encounter a higher proportion of infective hyphae from germinating spores of *A. laevis*. Obviously, any factor that changes the relative infectivity of propagules of different species of VAM

fungi would affect the development of fungal structures by each of the species present. Thus, the formation of these fungal structures by any one species in a field soil will depend on the relative number of its infective hyphae present near roots (Abbot *et al.*, 1991).

Infection by VAM fungi is usually initiated from hyphae growing from soil-borne propagules or from neighbouring infected roots. Recognition events must occur between the two organisms, the first visible sign being the formation of appressoria on the root surface. Infections develop within the root cortex, with hyphae growing longitudinally between cells, and with intracellular development of arbuscules. Vesicles containing large amounts of lipids are formed later in the maturation of an infected root. As the infection spreads within the root, extramatrical hyphae grow out into the soil (Harley *et al.*, 1983). However, the patterns of infection vary depending on the VAM species and the host involved. Boyetchko and Tewari (1990) concluded that the pattern of root colonization by *Glomus dimorphicum* was influenced by the host genome and that the fungal morphology in the roots was variable, and thus not diagnostic for mycorrhizal species. The spread of infection measured as the fraction of root length infected usually followed a sigmoid curve and was under host control. The length of the lag phase, the slope of the phase of rapid spread, and the height of the plateau vary depending on the host-fungus combination, density of inoculum in the soil, and enviromental conditions (Tinker, 1975).

2. 3. Role of VAM in Agriculture

The role of VAM in enhancing phosphate uptake and improving plant growth is widely recognized (Hayman *et al.*, 1981; Abbot and Robson, 1982). VAM fungi, found in most agricultural crops except sugar beet and the brassicas, can increase growth several-fold under certain conditions, especially where soil P is a limiting factor. It is generally believed that VAM fungi increase nutrient uptake primarily by shortening the distance that nutrients must diffuse through the soil to the root (Abbott and Robson, 1982).

Hattingh *et al.* (1973) found that mycorrhizal hyphae could intercept ^{32}P placed 27 mm from a mycorrhizal root, whereas it remained unavailable to non-mycorrhizal roots. Owusu-Bennoah and Mosse (1979) extended this observation and the similar results of Rhodes and Gerdemann (1975), by showing that the radius of the depletion zone for phosphorus around mycorrhizal onion roots was twice that for roots of non-mycorrhizal onions. Hence, mycorrhizal hyphae increase the volume of soil available to the plant for nutrient uptake (Figure 1).

Differences have been observed between mycorrhizal and non-mycorrhizal roots also in their rates of absorption of nutrients from soil solution, where diffusion does not limit absorption. Mycorrhizal roots absorbed more P or Zn per gram of root than did non-mycorrhizal plants (Skinner and Bowen, 1974; Faber *et al.*, 1990).

While external hyphae are important in nutrient uptake by mycorrhizal roots, the effects of nutrient concentration in soil solution and of other factors on the absorption of nutrients by the hyphae of the VAM fungi have not been studied (Tinker 1978; Hayman

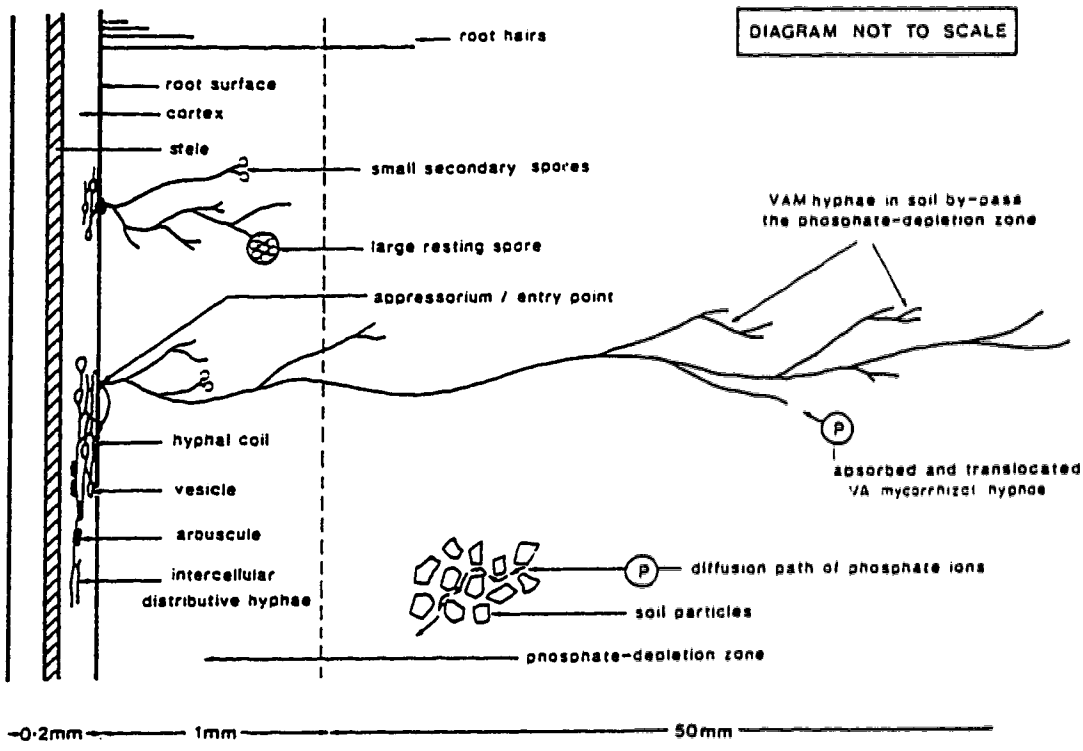

Figure 1. Diagrammatic representation of a VAM fungal root and the chief mechanism by which it is believed to enhance the uptake of phosphate from soil. The hyphae extend well beyond the root hair and P-depletion zones and translocate the nutrient ions directly to the root cortex, thus bypassing the slow diffussion paths of phosphate in soil. Adapted from Hayman, 1982.

et al., 1981). Such studies would permit comparisons to be made between the limiting nutrient concentrations in soil solution for uptake by intact plant roots.

Increases in plant growth and yield resulting from inoculation with VAM fungi have been observed in several field crops. Khan (1975) reported increased growth and yield of mycorrhizal wheat plants in low-P soils compared to non-mycorrhizal controls. Grain yield was increased three-fold by the fungus, indicating that VAM stimulated growth and increased yield in field soils deficient in P. He concluded that the mycorrhizal effect consisted primarily of improving the supply of P. Field studies by Khan (1972) have shown that the subsequent growth of maize seedlings that were already mycorrhizal when transplanted into field soil containing little P was considerably better than that of non-mycorrhizal controls. Mosse and Hayman (1971) and Mosse *et al.* (1977) reached similar conclusions with mycorrhizal maize and onion seedlings, respectively.

Owusu-Bennoah and Mosse (1979) reported that onions, alfalfa, and barley plants inoculated with VAM fungi had increased growth and yield subsequently but that alfalfa and onions benefited most from inoculation in soils with most available P whereas barley responses were confined to those with less P available. Faber *et al.* (1990) observed that mycorrhizal citrus plants had increased growth and yield and showed fewer symptoms of zinc deficiency in soils than the non-mycorrhizal plants.

Although most of the work done with VAM has concentrated on their role in plant nutrition, there is now increasing interest also in

drought resistance of mycorrhizal plants (Allen and Boosalis, 1983; Busse and Ellis, 1985; Ellis *et al.*, 1987). VAM fungal infection has been reported to increase nutrient uptake in stressed plants (Busse and Ellis, 1985), lower stomatal resistance (Stahl and Smith, 1984), to enable plants to use water more efficiently, and to increase root hydraulic conductivity (Graham and Syvertsen, 1984). Few studies, however, have compared the effects of different mycorrhizal inocula to see if some are more efficient than others in increasing drought tolerance, and still fewer reports are available on the effects of water stress on the fungi themselves.

It has also been shown that VAM inoculation of field crops is not always beneficial to the host plant. In some instances, VAM inoculation significantly reduced host plant growth, suggesting a parasitic effect that limits carbohydrate availability in the host (Bethlenfalray *et al.*, 1982). These results emphasize the variation in the effectiveness of different VAM fungi (Abbott and Robson, 1978; Carling and Brown, 1980) and the fact that VAM fungi are not always beneficial to their host. Simpson and Daft (1990) observed that both *Glomus monosporum* and *Acauolospora* sp. inoculation resulted in growth reduction in maize and sorghum despite good root infection levels. The VAM fungi species did not increase drought resistance of the host plants, either in terms of maintaining growth under stress and speeding recovery from wilting or by more efficient use of water. The authors suggested that before VAM inoculum is introduced into drought-prone soils, the ability to cause infection and to sporulate in such an enviroment, together with any

effects on plant growth and survival, must be investigated under realistic conditions.

2. 4. VAM and Carbohydrate Physiology

Mycorrhizal fungi invade cells of host roots which tolerate and maintain the presence of what can be a large volume of fungal tissue. In spite of the repercussions that this must have on root metabolism together with the carbohydrate drain on the plant, the formation of VA mycorrhizae can influence positively several aspects of plant physiology: carbohydrate physiology, phosphate nutrition, uptake of trace elements and water, hormone production, nitrogen fixation, and resistance to plant diseases (Hayman, 1983).

Trappe (1973) and Gianinazzi *et al.* (1983) observed that the carbon requirements of VAM fungi are supplied by photosynthate from the host plant. Snellgrove *et al.* (1982) concluded that the demand on host photosynthates depends upon the amount of fungus associated with the root and its metabolic activity. They also noted that increase in metabolic activity in infected roots, together with fungal biomass production and respiration, is a cost to the host plant and could result in yield reduction because up to 10% of photosynthates are exported to mycorrhizal plant roots. Gianinazzi (1983) stated that the form of carbon and its mode of transfer from plant to fungus are as yet unknown but, since the host cells always outlive the fungus, the transfer must be biotrophic and must take place across the living interface between the two organisms.

VAM fungi convert the transferred host photosynthates into specific fungal compounds that cannot be utilized readily by the

host plant. Most of these appear to be lipids or glycogen (Gianinazzi, 1983; Hayman, 1983). Lipids are particularly abundant in mature arbuscules, vesicles, and mature hyphae but not in young mycelium and arbuscules. Glycogen granules usually are associated with these lipid-containing structures and are also found in young vacuolated hyphae and the finest arbuscular branches (Kinden *et al.*, 1975).

The way in which carbon compounds are transported to other parts of the fungal mycelia that are developing in soil around mycorrhizal roots is not known. One possibility is that protoplasmic streaming, which can be bi-directional in fungal hyphae, could carry glycogen particles or lipid globules down a carbohydrate concentration gradient toward external hyphae (Losel *et al.*, 1979; Gianinazzi *et al.*, 1981; Hayman, 1983).

Host-fungus competition for carbon has been suggested as a cause of plant growth supression in VA mycorrhizal plants (Buwalda *et al.*, 1982; Stribley *et al.*, 1980). Mycorrhizae incorporate greater amounts of carbon, derived from photosynthates, than non-mycorrhizal roots. Starch grains disappear from cells during active fungal development, and carbon loss through respiration can be considerably higher. The loss of photosynthates from mycorrhizal plants can be compensated by increased photosynthesis (Gianinazzi *et al.*, 1983).

2. 5. Phosphate Nutrition

One of the major benefits of VA mycorrhizal association to the host plant is the increased uptake of nutrients such as phosphorus (P), zinc (Zn), copper Cu), nitrogen (N), and sulphur (S). Several authors have reported a close correlation between a VAM fungus and P nutrition of the host (Sanders and Tinker 1973; Menge *et al.*, 1978). In most instances, only 20-30% of applied fertilizer P is utilized by the crop in the first year and the remaining amount becomes fixed in the soil. The residual P may be continually used by the plant for up to 15 years after application. Because P is a highly immobile plant nutrient, VAM fungi play an important role in the recovery of fixed P which may be largely unavailable to the plant (Menge *et al.*, 1978).

VAM fungi are able to increase P uptake from soil and improve plant growth in P-deficient soils (Mosse 1973; Abbot and Robson, 1982). Generally, VAM infection is greatest in soils low in P, with infection decreasing with increasing P fertilization rates (Safir and Dunway, 1982; Hoefner *et al.*, 1983) Many researchers agree that plant growth responses to VAM infection are greater when plants are supplied with lower P rates than with high P rates (Lambert *et al.*, 1980; Hayman, 1983). It is possible, however, that at least some mycorrhizal fungi species can tolerate higher soil P levels (Hayman, 1983).

In most instances, increased phosphate uptake is the primary cause of growth and yield enhancements in mycorrhizal plants. Mycorrhizal roots have different phosphate absorption kinetics and lower threshold values than non-mycorrhizal roots. The external hyphae around mycorrhizal roots explore a larger volume of soil and

absorb available phosphate beyond the depletion zone of the root (Gianinazzi *et al.*, 1983). The extraradical mycelium has been observed at a distance of at least 1 cm beyond the root (Sanders and Tinker, 1973).

Two theories have been proposed regarding P transfer from mycorrhizal fungi to the host. Phosphorus is released into the host upon digestion of the arbuscule (Cox and Tinker, 1976), or the P that accumulates in the external fungal hyphae is translocated to the internal mycelium by a well developed transport system and transferred to the host tissues mainly across the intercellular arbuscules (Gianinazzi-Pearson and Gianinazzi, 1983). The walls of the arbuscules are composed primarily of glycolipids while those of vesicles and hyphae are predominantly made of chitin. It is believed that the glycolipid component facilitates the bi-directional flow of nutrients more readily than the chitinous component. The activity of various enzymes at the host-fungus interface supports the theory that nutrient transfer occurs in a living system (Nemec, 1981).

Inorganic P is released upon degradation of the polyphosphate by a polyphosphatase enzyme as it reaches the arbuscule (Callow *et al.*, 1978). Enzymes involved in the polyphosphate degradation, such as endopolyphosphatase and exopolyphosphatase, have shown greater activity in mycorrhizal than in non-mycorrhizal roots (Capacio and Callow, 1982). Polyphosphate glucokinase activity was detected in the external hyphae of the mycorrhizal roots, glucose-6-phosphate being the intermediate product. It is believed that polyphosphate glucokinase has a potential role in the transfer of glucose from the host to the fungus but, because its activity was detected in the

external hyphae, its complete role is not well understood (Capaccio and Callow, 1982).

It has been suggested that a polyposphate kinase enzyme liberates ATP, which can be used in the translocation of inorganic P across the host-arbuscule interface. Marx *et al.* (1982) support the hypothesis that P is transferred across the host-arbuscule interface via an active transport mechanism. Plasmalemma-bound-ATPase activity of the host was concentrated around the arbuscule branches when VA mycorrhizae developed in the root cells. The distribution of the ATPase activity was not detected in the very young or degenerating arbuscules. It was suggested, therefore, that nutrient exchange occurs across the interface in a living system.

2. 6. Nitrogen Nutrition

Nitrogen uptake, translocation, and transfer via VAM fungi have been studied by several researchers. Reeves (1992) reported that nitrogen (N) can be transferred from N-fixing legumes via VAM fungi to associated non-fixing plants in the greenhouse. She observed that this transfer can occur only where mineral N is applied shortly before harvest, and hence is readily available. It is yet to be demonstrated that VAM mediate N transfer when soil N is limiting, a condition under which most traditional crops are grown. Using ^{15}N-enriched soils to distinguish between the uptake of soil-derived and atmospherically derived N in maize (*Zea mays* L.) grown in the presence or absence of VAM, Reeves (1991) observed that VAM fungi infection did not result in transfer of fixed N or soil N from bean (*Phaseolus vulgaris* L.) to maize, despite a VAM-stimulated

increase in N fixation in beans. In fact, beans were more competitive for soil N when infected by VAM fungi. N content in beans increased 75%, while in maize there was a 22% decrease.

Hamel *et al.* (1991) studied the effects of mycorrhizal inoculation on transfer of ^{15}N from soybean to maize, using three *Glomus* species and non-fumigated and fumigated soils. They showed that VAM enhanced the transfer of ^{15}N from soybean to maize but only in non-fumigated soils. High ^{15}N transfer was associated not only with high mycelium density in the soil but also with low soil microbial carbon, suggesting that the effects of mycorrhizal fungi on soil microbial populations may be an important factor affecting N transfer between mycorrhizal plants. Van Kessel *et al.* (1985) observed that the VAM fungus *G. fasciculatus* enhanced N transfer from soybean to maize. The mechanism of transfer from the donor plant to the receiving plant was by direct active transport. Haystead *et al.* (1988) showed that ^{15}N applied as $(^{15}NH_4)_2SO_4$ to white clover (*Trifolium repens* L.) was actively transferred via a hyphal pathway to companion ryegrass (*Lolium perenne.*).

2. 7. Uptake of Zinc and Sulphur

Zinc deficiency in agricultural crops is becoming one of the common micronutrient deficiencies. Faber *et al.* (1991) reported that there was increased zinc uptake via the hyphal pathway by corn plants grown in low Zn soil. Cooper and Tinker (1978) reached the same conclusion for white clover. They suggested that Zn translocation in hyphae is an active process, under metabolic control, and that the amount transported may be controlled by the

demand of the host plant rather than by the amount of absorbing mycelium. They also suggested that there may be an array of secondary influences by which the symbiosis may affect plant nutrition, including altered stomatal response and xylem transport.

Uptake and translocation of sulphur have been shown to occur in mycorrhizal roots. Gerderman and Gray (1973) showed that roots of mycorrhizal red clover absorbed and transferred more ^{35}S than those of non-mycorrhizal plants. Tinker and Cooper (1978) observed the same in white clover and onions.

2. 8. Uptake of Herbicides

There have been a few reports on the influence of VAM on the fate of herbicides in soils and plants. Busse and Ellis (1987) suggested that *G. fasciculatum* could enhance uptake of residual soil atrazine into soybean plants. Nelson and Khan (1992) showed that *Glomus* spp. were able to remove ^{14}C-atrazine from soil and transfer it via a hyphal pathway into corn plants. Nelson and Khan (1990) reported that hyphae of *G. intraradices* and *G. vesiculiferium* were able to remove bound ^{14}C-fonos and transport it to onions. They concluded that, under field conditions, endomycorrhizal infection may greatly increase the bioavailability of soil-bound pesticide residues to plants.

However, there are no reports in the literature on the role of VAM fungi in uptake of soil-applied herbicides via the root system of weed plants.

2. 9. Production and Application of VAM Fungi

Exploitation of VAM fungi for agricultural use is dependent on the production of commercially viable inoculum on plant hosts or on manipulating agricultural systems to develop and exploit indigenous VAM populations (Simpson and Daft, 1990). Miller and Evans (1988 and 1990) showed that in undisturbed or zero-tilled soils, VAM enhanced P uptake by maize more than in disturbed soils.

VAM fungi are considered obligate symbionts that cannot be grown in pure culture (Hepper, 1984), so the difficulties involved in producing, storing, and applying VAM fungi in field crop systems preclude any easy usage of the fungus even when growth benefits are likely (Hayman, 1987).

Of the various techniques devised for introducing VAM fungi inoculum into field-grown crops, the use of pre-inoculated transplants is the simplest (Hayman, 1987). Adhesives such as methyl-cellulose inoculated with VAM fungal spores that have been concentrated by wet-sieving can be used to suspend germinated seed in a slurry that then is applied in seed furrows. This procedure has been successful in field inoculation of red clover (Hayman *et al.*, 1981), but it is impractical with smaller-seeded crops (Hayman, 1984). The use of seeds stuck onto pellets of soil inoculum has been successful with some crops (Hayman, 1981). Increasing the density of VAM fungi *in situ* by growing a heavily mycorrhizal crop in a rotation system is time-consuming. Top soil transferred into an area as VAM inoculum also introduces a new microflora that may contain plant pathogens.

The large quantities of VAM inoculum needed for field inoculation usually are raised in pot cultures, which frequently become contaminated by other VAM fungi (Hall, 1977) or by other contaminants that may be pathogenic to plants (Hayman, 1987). Inoculum also has been produced successfully in aeroponic cultures, resulting in well colonized root systems and proliferation of spores (Hung and Sylvia, 1988). If plants could be inoculated without soil, contamination of VAM fungi cultures by root pathogens, hyper-parasites, or other VAM fungi could be eliminated (Hung *et al.*, 1991). Aseptic, viable VAM fungi spores have been produced successfully (Tommerup and Kidby, 1979), and several hydrogels can be used as sticking agents for direct inoculation of VAM fungi spores onto host plant roots (Hung *et al.*, 1991), thereby avoiding contamination. On the other hand, deliberate contamination of VAM inoculum with compatible micro-organisms that are beneficial to plants could be a useful innovation (Hayman, 1987). Since the stock cultures are raised in sterilized soils, selected micro-organisms could be introduced and established before invasion and competition from aerial contaminants becomes intense. Bacteria that dissolve insoluble phosphates, produce growth-promoting compounds, or are antagonistic to specific plant pathogens might be introduced into the rhizosphere this way (Hayman, 1987). One should take into consideration the fact that some micro-organisms may inhibit germination of specific VAM fungi and others may stimulate it (Tommerup, 1985).

Additional problems in large scale use of commercial VAM as inoculants in field crops include selection of VA endophytes suitable

for particular host-soil-climate combinations and possible competition with the indigenous mycorrhizal fungi (Hayman, 1981). Also, the inoculant VAM fungi may not persist in the soil at a high enough inoculum potential to continue uptake of nutrients after inoculation. They also may remain dormant after inoculation to the host plant (Abbott and Robson, 1982).

2. 10. Root Uptake of Herbicides

The mode of action of a number of herbicides involves root absorption, followed by translocation to leaves. When herbicides such as substituted ureas or triazines are applied to roots they apparently move into the xylem elements (apoplasm) and are carried in the transpiration stream to leaves (Martin *et al.*, 1976; Ashton *et al.*, 1981). Root tissue constitutes a large surface area of absorption of soil-applied herbicides, and the single-celled root hairs increase this surface area (Duke, 1985). Besides anchoring the plant to the soil, the primary function of the root is to absorb water and nutrients from the soil. During growth, plants require the influx of large amounts of water. The absorption of this water occurs primarily at the root hair zone. Herbicides dissolved in water come into contact with the root surface and can be absorbed along with the water. Herbicide molecules in contact with the root, from the meristematic region through the functioning root hair zone, appear to move into the root by simple diffusion (Duke, 1985) or by mass flow (Kirkwood, 1991).

Herbicides normally can enter into roots by three routes: apoplast, symplast, and apoplast-symplast. The apoplast route

involves movement in the cell walls to the xylem. This route appears to require that the herbicide pass the Casparian strip, a water tight barrier in the cell walls of the endodermis separating the cortex and the stele (Ashton *et al.*, 1981; Waisel *et al.*, 1991). The symplast route involves initial entry into cell walls and then into the cytoplasm of the cells of the epidermis, cortex, or both. The herbicide remains in the cytoplasm and subquently passes into the endodermis, stele and phloem by means of plasmodesmata (Ashton *et al.*, 1981). The apoplast-symplast route is identical to the symplast route but the herbicide may re-enter the cell walls after passing the Casparian strip and then enter the xylem. The chemical and physical properties of each herbicide primarily determine which route is followed (Ashton *et al.*, 1982; Kirkwood *et al.*, 1991).

2. 11. Interaction of VAM Fungi with Herbicides

Selective herbicides are commonly used to control weeds in arable crops, because better crop growth, higher yields and less physical damage occur in crops under chemical weed control programmes than under tillage programmes (Nemec and Tucker, 1983). Like soil and foliage-applied insecticides or fungicides, most herbicides have the potential to affect the soil microflora (Pope *et al.*, 1978). Recent studies have shown that some herbicides do affect the development of symbiotic host-beneficial endomycorrhizal fungi in soil and plant roots (Holt *et al.*, 1981). These authors, for example, showed that paraquat influences the development and efficacy of the mycorrhizal fungus *G. fasciculatus* on white ash seedlings. Paraquat applied at 2 kg/ha on a very sandy soil

significantly reduced the amount of mycorrhizal hyphae and the number of chlamydospores formed by *G. fasciculatus*. Paraquat at 0.5 kg/ha tended to promote the development of hyphae but the increase was not statistically significant. Nemec and Tucker (1983), on the other hand, reported that bromacil, diuron, and trifluralin had no effect on citrus root colonization by VAM fungi. High rates of simazine and paraquat damaged the plants and reduced fungal development. Presumably, the reduced root colonization was due to the herbicidal reduction of photosynthesis and carbon supply to the roots and not to a selective effect against the fungus. Ocampo *et al.* (1988) concluded that plant growth and VAM infections of pea roots inoculated with *G. mosseae* were decreased by MCPA applied at high doses. They suggested that the VAM fungi were affected not only through the plant but also directly by the application of the herbicide.

Burpee *et al.* (1978) observed that alachlor and trifuralin at the recommended rate of 2 kg/ha, incorporated into the soil prior to planting, did not affect mycorrhizal development, shoot weight, or phosphorus accumulation in soybeans. However, at 4 kg/ha both herbicides inhibited root growth and reduced shoot weight, and suppression of mycorrhizal infection and development occurred. Ocampo *et al.* (1985) studied the effects of the carbamate herbicides chlorpropham, sulfallate, and phenmedipham which inhibit cell division and plant growth. Foliar application of phenmedipham decreased root concentration of total and reducing sugars and decreased fungal metabolism 48 h after application. However, none of the three herbicides affected the amount of VAM infection at the

end of the experiment. The herbicides decreased plant growth when applied to the soil but, when they were applied to the foliage, only phenmedipham applied at high concentrations decreased plant growth.

Schwab *et al.* (1982) reported that simazine applied at less than 2 ppm increased the mycorrhizal formation and development in *Chenopodium quinona*. There was no increase in VAM formation when simazine was applied at a dose higher than 2 ppm. Smith *et al.* (1981) reported that paraquat and diquat had no measurable effect on VAM endophyte spore abundance. There was a slight trend to lower VAM endophyte spore numbers at high rates of application of diallate and triallate but not for diuron and trifluralin. Percent infection declined at high rates of diallate and led to lower mycorrhizal root weights. The phosphorus content of the shoots was also reducd by diallate. High doses of diallate, diuron, triallate, and trifluralin reduced most parameters of plant growth more than mycorrhizal parameters. The authors concluded, therefore, that at normal application rates these herbicides are unlikely to affect endomycorrhizal formation or function adversely.

It can be concluded, therefore, that herbicide application can have varied effects on VAM formation and function. Mycorrhizal responses are often related to the biochemical mechanisms of action of the herbicide and its concentration in the soil. Most herbicide concentrations greater than field rates cause interruptions of VAM activities (Moorman, 1989).

3. Materials and Methods

3. 1. Plant Material

Wild oat, green foxtail, and white clover seed was obtained from the University of Alberta Edmonton Research Station. Seeds were planted in 15-cm diameter plastic pots in the greenhouse or in 150 by 15 mm petri dishes in a growth cabinet. Wild oat and green foxtail plants grew rapidly and established extensive root systems. White clover plants grew slowly and did not establish an extensive root system.

3. 2. Vesicular-arbuscular Mycorrhiza (VAM) Source and Culture

3. 2. 1. Inoculum Production

Vesicular-arbuscular mycorrhiza (VAM) inoculum species *Glomus intraradices* (originally obtained by Dr. J. P. Tewari from the University of Florida International Culture Collection of VA Mycorrhizal Fungi under accession no. 208) consisting of infested soil and alfalfa root pieces was multiplied in the greenhouse for 10 months and used in the first set of experiments. This inoculum was later discarded as it was found to be heavily infested with harmful fungal pathogens such as *Pythium* and *Rhizoctonia*. Pure VAM inoculum obtained from David McArthur (a graduate student in the Department of Plant Science) then was multiplied using red clover as the host. The inoculum was multiplied in 15-cm plastic pots containing autoclaved soil mixture (sand: clay loam, 3:1). Each pot

received 50 g of VAM inoculum put in a shallow depression in the plastic pot and covered with potting medium. Red clover seeds then were planted. The seedlings were thinned to six per pot after 14 days. Each pot received 100 ml full-strength Hoagland nutrient solution without P weekly, in addition to daily watering. This inoculum source was used in all subsequent experiments.

3. 2. 2. Determination of Spore Density in the Inoculum

Five grams of soil was mixed with tap water and stirred for 2 min. The slurry was put on a Waring blender, and blended intermittenly for 30 sec at a low speed to dislodge spores. The mixture then was decanted successively onto two sieves with pore sizes 425 and 45 μm, and washed with tap water for 2 min. The residue on the 45-μm sieve was washed into a 50-ml beaker. The spores in this washing were rescued using a modification of the sucrose gradient centrifugation (J. P. Tewari, personal communication). A 10-ml aliquot of 60% sucrose solution was pipetted below a 10-ml aliqout of 20% sucrose, creating a gradient with an interface. Aliquots of the spore mixture (10 ml) were pipetted onto the gradient interface and centrifuged at 2500 rpm for 3 min using a desktop centrifuge. The layer of VAM spores at the interface then was pipetted onto a 45-μm sieve and rinsed with tap water to remove sucrose. The spores were collected onto a Whatman#1 filter paper under suction using a millipore apparatus (Figure 2). Fungal spore counts were done under a stereo microscope. Four replicate samples were used to obtain a reasonable estimate of the average spore density in the soil mixture.

Figure 2. Millipore apparatus for collection of VAM spores onto a filter paper.

3. 3. Susceptibility of VAM-Infected and Non-Infected Plants to Herbicides.

3. 3. 1. Plant Growth

In the experiments in which the phytotoxicity of soil-applied herbicides to VAM-infected and non-infected plants was compared, plants were grown in a conventional greenhouse at temperatures of 23 C/20 C day and night, with a day length of 16 h. The light source was high pressure sodium 400 W bulbs that produced an average photon flux density of 450 mmol m^{-2} s^{-1} measured with a Li-Cor quantum meter, model Li-188.

Wild oat and green foxtail seeds were planted in individual plastic pots filled with an autoclaved soil mixture (sand: clay loam, 3:1). The soil was autoclaved twice for 1 h at 112 C with a 24-h interval, to kill soil-borne microbes. The seeds were surface-sterilized in 0.5% sodium hypochlorite for 20 min and rinsed with several changes of distilled water to kill seed-borne contaminating fungi. In the first run of the experiments, each pot received 100 g of VAM inoculum in the form of infested soil and alfalfa root pieces. When the experiments were repeated, each pot received inoculum from infested soil and red clover root pieces. The inoculum was spread on the surface of the potting medium and then covered with more potting medium. The plants in each pot received 100 ml full strength Hoagland nutrient solution without P (Hoagland and Arnon, 1952) once a week and were watered twice daily. In addition, green foxtail plants in the third run of the experiment received 100 ml of 5 g/L 20-20-20 complete fertilizer four times in the entire period

of the experiment, in order to alleviate the serious P deficiency observed in the earlier experiments. Eight days after emergence, wild oat plants were thinned to 10 uniform plants per pot. Green foxtail plants were thinned to 10 uniform plants after 10 days.

3. 3. 2. Herbicide Source and Application

Atrazine[1] was applied to the soil surface in 20 ml water per pot, using a 650-ml hand sprayer. The amounts applied were equivalent to 0.3, and 0.5 kg ai/ha for wild oats, and 1.0 and 1.5 kg ai/ha for green foxtail. In all three runs for the wild oat plants and the first two for green foxtail, plants were at the 3-leaf stage when the herbicide was applied. The herbicide was applied at the 4-5 leaf stage for green foxtail in the third run of the experiment. Care was taken to ensure that none of the spray was intercepted by the leaves or the stems. In wild oat plants, injury symptoms occurred 7 days after herbicide application for both inoculated and uninoculated plants. In green foxtail plants, injury symptoms occurred 6 days after herbicide application.

Similarly, diclofop-methyl emulsion[2] was applied to the soil surface in amounts equivalent to 0.28 and 0.37 kg ai/ha, in 20 ml water per pot` at the 3-leaf stage for both wild oats and green foxtail plants. The herbicide was applied at the 4-5 leaf stage for green foxtail plants in the third run of the experiment. In wild oats, injury symptoms occurred 8 days after plant growth for the first and the third run of the experiments for both inoculated and

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[1] Commercial product 90 WP (Ciba Geigy)
[2] Commercial product 284 g/L (Hoechst)

uninoculated plants. In the second run, injury symptoms occurred after 14 days. In green foxtail plants, injury symptoms occurred 4 days after herbicide application for the first and the second experiment. In the second run, injury symptoms were observed after 8 days in the third run of the experiment. Herbicide injury levels on plants were scored using a 0-9 scale. A score of zero indicated no injury and 9 represented death of the plant.

The herbicide rates for all the experiments were decided on the basis of preliminary dose-response experiments performed earlier. The aims of these experiments were to have herbicide application rates that would provide 40 to 50% growth supression of the selected weeds.

3. 3. 3. Plant Harvest

Plants were harvested 6 weeks after planting in the first run of the experiments, and 8 weeks after planting in the second and third runs. Shoots were dried for 24 h at 65C and dry weights were recorded. Part of the root system was cleared and stained and the percentage of VAM infection was determined. Other parts were cut to 1-cm lenghts and stored in bottles containing FAA solution (5% formalin, 5% acetic acid, 45% ethanol, 45% water) until they were cleared and stained.

The experimental design was a randomized complete block with four replicates.

3. 4. Uptake of ^{14}C-atrazine by VAM-infected and Non-infected Roots of White Clover and Green Foxtail

3. 4. 1. Plant Growth and VAM Inoculation

In the growth cabinet, plants for ^{14}C-atrazine uptake experiments were grown for 72 days in a day/night temperatures of 22C/20C initially, later changed to 24 C/22 C, and a relative humidity of 70/70%. A 16-h photoperiod of 500±5 mmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) was maintained throughout the growth period. The light source was a combination of Sylvania cool-white (VHO) and pink wide spectrum 3-m bulbs.

Green foxtail and white clover seeds were surface-sterilized with 0.5% sodium hypochlorite solution for 20 min, rinsed with several changes of distilled water, and then sown in autoclaved sandy loam soil contained in the upper compartment of specially modified disposable petri dishes (Figure 3). The seedlings were thinned to one per petri dish 15 days after plant emergence. Occasionally plants were pruned to bring them to uniform size. In particular, the main stem of green foxtail plants was cut down several weeks before harvesting to stimulate lateral growth and production of young actively growing shoots.

The treatment vessels were 150 by 15 mm sterilized disposable petri dishes modified to allow insertion of polyester fiber screens into the dish to form two soil compartments (a method adopted with some changes from Nelson and Khan, 1992). The soil compartments were separated by two different polyester fiber screens. A 44-μm plain mesh screen prevented root penetration but

Figure 3. Green foxtail plants growing in specially constructed petri dishes.

allowed hyphal penetration and a 1000-μm screen allowed both root and hyphal penetration. The screens were sealed to plexiglass support bars and the support bars in turn were sealed to the petri dishes with GE-sealant (a silicone adhesive) to ensure that the roots and the hyphae had to pass through the screens in order to make contact with the lower soil compartment (Figure 4). The upper portion of the petri dish, therefore, was sealed to the screens but the lower portion of the dish was removable. The petri dishes were sterilized with 5% sodium hypochlorite for 12 h and rinsed with several changes of distilled water. After drying, 70% alcohol was applied to kill contaminating fungi. Moistened soil mixture (sand: clay loam, 3:1) was autoclaved twice at 112 C for 1 h with a 24-h interval, to kill soil-borne harmful fungi. The soil mixture was placed in the lower and upper compartment of the petri dishes. Vessels were tapped to cause the soil to settle and to ensure that at least a 2-mm air gap separated the screen from the soil in the lower compartment. The VAM inoculum was a mixture of soil and root fragments of red clover stock culture, harvested 6 months after planting. The roots were cut into 1-mm fragments and thoroughly mixed with the soil particles to ensure uniform soil inoculum. The sucrose density gradient method described earlier was used to assess the number of spores per gram of soil. Ten grams (dry weight) of soil inoculum was placed in the rooting zone of the upper compartment of the treatment vessels. Distilled water was applied regularly to the upper compartment. Water levels were monitored carefully to minimize attack by harmful fungal pathogens, and to prevent errors due to capillary rise or by leaching of the radio-

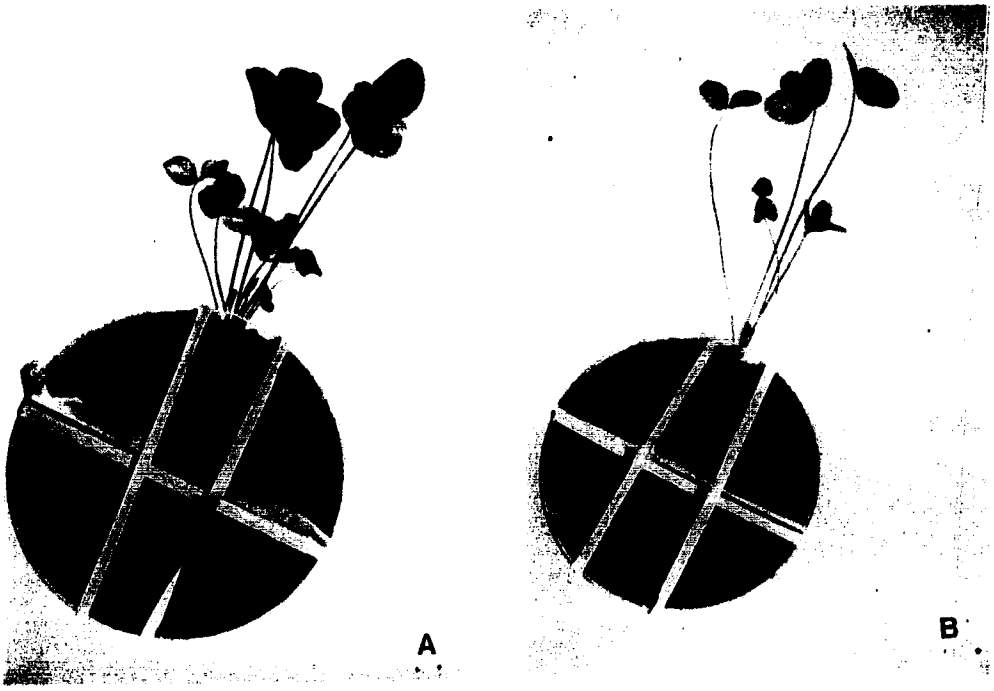


Figure 4. White clover plants growing in specially constructed petri dishes.
A. Plant roots restricted to the upper compartment by 44 µm screen.
B. Plant roots penetrating the 100 µm srceen from upper to lower compartment.

labelled material from the lower soil compartment.

In addition, the petri dishes received weekly applications of 25 ml full strength Hoagland nutrient solution without P (Hoagland and Arnon, 1952). Green foxtail plants received 25 ml of 5 g/L 20-20-20 complete fertilizer four times during the experimental period in order to alleviate the serious P deficiency problems observed in the greenhouse-grown plants.

3. 4. 1. ^{14}C-atrazine Application

The labelled herbicide was a donation from Ciba Chemical Company in North Carolina. The ^{14}C-atrazine (5 mg) had a specific activity of 20.7 mCi/mg, and a chemical purity of 97%. It was dissolved in 4 ml acetone to make a stock solution. To prepare treatment solution, 2.2 ml stock solution was pipetted into a 125-ml flask and 50 ml distilled water was added. This treatment solution was used in the first set of experiments. Acetone evaporated in the remaining stock solution and the dry ^{14}C-atrazine residue was re-dissolved in 0.5 ml acetone and made up to 50 ml treatment solution with the addition of 49.5 ml of distilled water.

The lower soil compartments of the treatment vessels were treated with ^{14}C-labelled atrazine solution. Each treatment vessel received 1 ml atrazine treatment solution. A 1000-µl Eppendorf digital pipette with disposable tip was used to apply the aliquot in small droplets uniformly distributed on the surface of the soil in the lower compartment of the treatment vessels.

3. 4. 3. Plant Harvest

Half of the plant samples were harvested 3 days after herbicide treatment and the rest 6 days after treatment and 72 days after seed germination. Leaf, petiole, stem, upper and lower root tissues were carefully separated, and cut into small pieces. The roots were cut at the mesh screen and divided into those in the upper compartment and those in the lower compartment of the treatment vessels. They were rinsed in several changes of distilled water to remove adhering soil particles. The plant tissues were cut into small pieces and dried at 65 C to constant weight. Total dry weight then was recorded.

The experimental design was a randomized complete block with four replicates.

3. 4. 4. Determination of Radioactivity

Two samples weighing about 50 mg per sample of each plant tissue part were combusted to $^{14}CO_2$ in a OX-300 biological oxidizer. The combustion time was 3 min. The $^{14}CO_2$ was absorbed in 12 ml of Harvey carbon-14 cocktail absorbent contained in liquid scintillation counter vials. Samples were analysed in a Tri-carb Liquid Scintillation Spectrometer (LSS) 4000 series. Total radioactivity in the plant parts then was calculated from the total dry weight of the shoots and roots.

3. 5. Root Clearing, Staining, and Infection Assesment

Roots were cleared and stained by a method described by Philips and Hayman (1970). The roots that had been stored in FAA

were washed in tap water, placed in a glass beaker, and covered with 10% KOH. The specimens then were heated in an oven at 90C for 35 min. The KOH cleared the host cytoplasm and the nuclei and allowed stain penetration. The KOH was poured off after heating and the roots were rinsed in tap water until no brown colour appeared in the rinse water. Thereafter, the roots were bleached with alkaline H_2O_2 (3 ml ammonium hydroxide, 30 ml 10% hydrogen peroxide, 567 ml tap water) at room temperature for 10 min, rinsed in water and acidified in 1% Trypan Blue lactic acid staining solution (875 ml lactic acid, 63 ml glycerine, 63 ml tap water, 0.1 g Trypan Blue) for 5 min. The staining solution then was poured off and lactic acid destaining solution (same as the staining solution but without Trypan Blue) was used to remove excess stain. The roots then were placed on slides and VAM root colonization was assessed by the grid-line intersect method recommended by Giovannetti and Mosse (1980) using a stereoscopic microscope. One hundred counts per slide were made, on four slides from each replicate per pot or vessel. When more detailed observations were desired, the roots were examined under a compound light microscope and photographs of VAM infection patterns and structures were taken with an automatic camera attached to it.

4. Results and Discussion

4. 1. Atrazine and Diclofop-Methyl on Wild Oats and Green Foxtail

Greenhouse experiments were carried out to study the role of VAM in uptake of soil-applied atrazine and diclofop-methyl by roots of wild oats and green foxtail plants. The VAM-infected wild oats and green foxtail readily formed structures such as spores, hyphae, arbuscules, and vesicles within the root cortex. The level of root colonization (percent infection) of control plants (no herbicide applied) varied from 26 to 52% for wild oats and from 50 to 70% for green foxtail plants (Tables 1, 2, 3, 4). When atrazine solution was applied to the soil surface, and plants were harvested five weeks later, the levels of root colonization varied from 26 to 48% for wild oats and 48 to 61% for green foxtail (Tables 1, 3). When the plants were treated with diclofop-methyl, these levels ranged from 30 to 49% for wild oats and 45 to 67% for green foxtail (Tables 2, 4).

The level of root colonization in VAM-infected herbicide-treated plants did not differ significantly from that in untreated VAM-infected plants (Tables 1, 2, 3, 4). Fungal structures such as spores, hyphae, arbuscules, and vesicles readily formed within the root cortex region of both control plants (no herbicide applied Figures 5, 6) and herbicide-treated plants (Figures 7, 8, 9, 10). These observations suggest, therefore, that the herbicide treatments had no observable effect on VAM fungal growth and development.

Table 1. Percent infection of wild oat plants inoculated with VAM. Roots were washed and sampled 5 weeks after application of atrazine solution to the soil surface, and roots were stored in FAA. They were washed in cold water, boiled in 10% KOH, stained in Trypan Blue, cleared, and the level of infection was assessed. Data are means of four replicates in each of three experiments.

	Atrazine on wild oats		
Herbicide treatment (kg ai/ha)	I	II	III
0	32	48	38
0.3	26	47	32
0.5	28	48	30

Table 2. Percent infection of wild oat plants inoculated with VAM. Roots were washed and sampled 5 weeks after application of diclofop-methyl emulsion to the soil surface, and roots were stored in FAA. They were washed in cold water, boiled in 10% KOH, stained in Trypan Blue, cleared, and the level of infection was assessed. Data are means of four replicates in each of three experiments.

	Diclofop-methyl on wild oats		
Herbicide treatment (kg ai/ha)	I	II	III
0	26	52	43
0.28	30	47	39
0.37	31	49	46

Table 3. Percent infection of green foxtail plants inoculated with VAM. Roots were washed and sampled 5 weeks after application of atrazine solution to the soil surface, and roots were stored in FAA. They were washed in cold water,boiled in 10% KOH, stained in Trypan Blue, cleared, and the level of infection was assessed. Data are means of four replicates in each of three experiments.

	Atrazine on green foxtail		
Herbicide treatment (kg ai/ha)	I	II	III
0	52	56	54
1	48	61	53
1.5	47	60	52

Table 4. Percent infection of green foxtail plants inoculated with VAM. Roots were washed and sampled 5 weeks after application of diclofop-methyl emulsion to the soil surface, and roots were stored in FAA. They were washed in cold water,boiled in 10% KOH, stained in Trypan Blue, cleared, and the level of infection was assessed. Data are means of four replicates in each of three experiments.

	Diclofop-methyl on green foxtail		
Herbicide treatment (kg ai/ha)	I	II	III
0	50	70	59
0.28	45	65	55
0.37	45	67	52

Figure 5. Structures formed by *Glomus intraradices* in roots of wild oats as seen under the light micrcoscope. Note differences in pattern of formation of structures. Photographs A and B are from control plants in the atrazine experiments; C and D are from the experiment with diclofop-methyl.

A. Spores, hyphae, and arbuscules. X 115.
B. Arbuscules. X 495.
C. Spores and vesicles. X 115.
D. Arbuscules, inside the root cortex. X 396.


A
B
C
D

Figure 6. Structures formed by *Glomus intraradices* in roots of green foxtail as seen under the light microscope. Note differences in pattern of formation of structures. Photographs A and B are from control plants in the atrazine experiment; C and D are from the experiment with diclofop-methyl.

A. Spore and hyphae. X 115.
B. Spores and hyphae. X 115.
C. Spores and arbuscules. X 115.
D. Vesicles and arbuscules inside the root cortex. X 115.

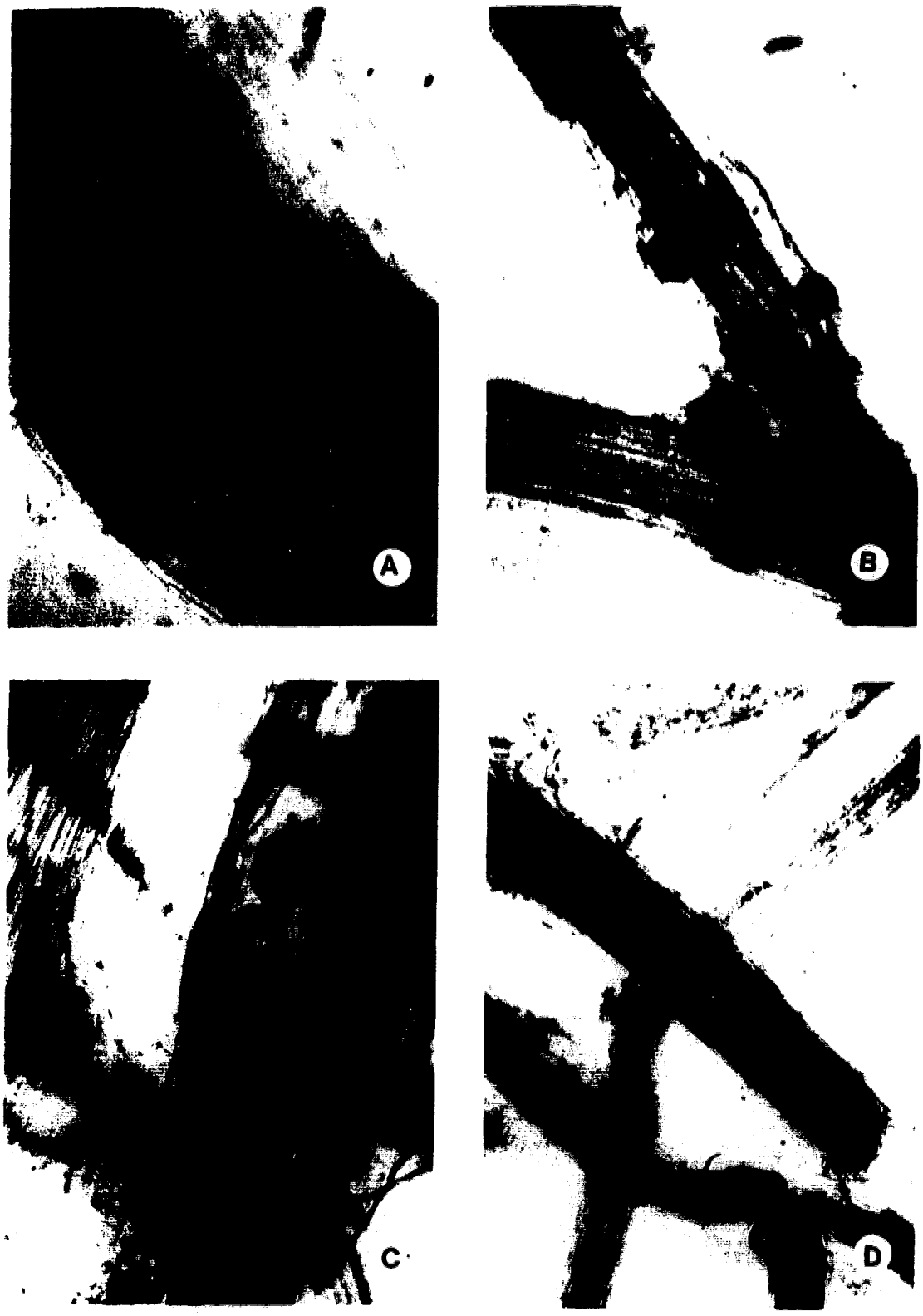

A
B
C
D

Figure 7. Structures formed by *Glomus intraradices* in roots of wild oats as seen under the light microscope. Note differences in pattern of formation of structures.

A. Spores, hyphae, arbuscules, and vesicles in wild oat roots treated with 0.3 kg ai/ha atrazine. X 115.
B. Spore and arbuscules in wild oat roots treated with 0.3 kg ai/ha atrazine. X 396.
C. Spores, arbuscules, and vesicles in wild oat roots treated with 0.5 kg ai/ha atrazine. X 115.
D. Arbuscules inside wild oat roots treated with 0.5 kg ai/ha atrazine. X 396.


A
B
C
D

Figure 8. Structures formed by *Glomus intraradices* in roots of green foxtail as seen under the light microscope. Note differences in pattern of formation of structures.

A. Spores, and arbuscules in a green foxtail root treated with 1.0 kg ai/ha atrazine. X 115.

B. Spores, arbuscules, and vesicles in green foxtail roots treated with 1.0 kg ai/ha atrazine. X 115.

C. Spores and arbuscules in green foxtail roots treated with 1.5 kg ai/ha atrazine. X 115

D. Arbuscules and vesicles inside the cortex of green foxtail roots treated with 1.5 kg ai/ha atrazine. X 396.

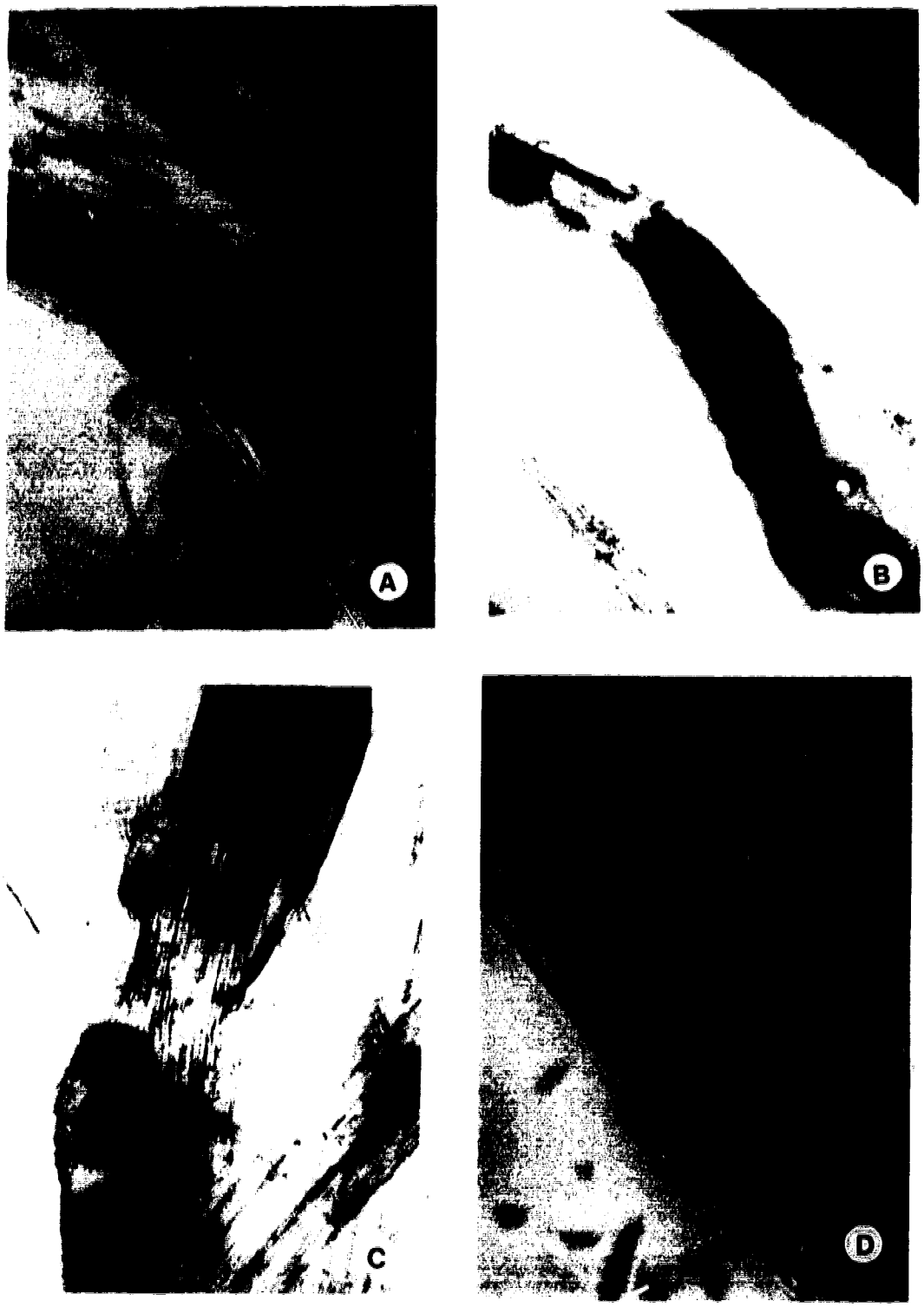

A
B
C
D

Figure 9. Structures formed by *Glomus intraradices* in roots of wild oats, as seen under the light microscope. Note differences in pattern of formation of structures.

A. Spores and vesicles in wild oat roots reated with 0.28 kg ai/ha diclofop-methyl. X 115.

B. Arbuscules inside the cortex of wild oat roots treated with 0.28 kg ai/ha diclofop-methyl. X 396.

C. Spores, arbuscules, and vesicles on wild oat roots treated with 0.37 kg ai/ha diclofop-methyl. X 115.

D. Arbuscules and vesicles inside the cortex of wild oat roots treated with 0.37 kg ai/ha diclofop-methyl. X 396.

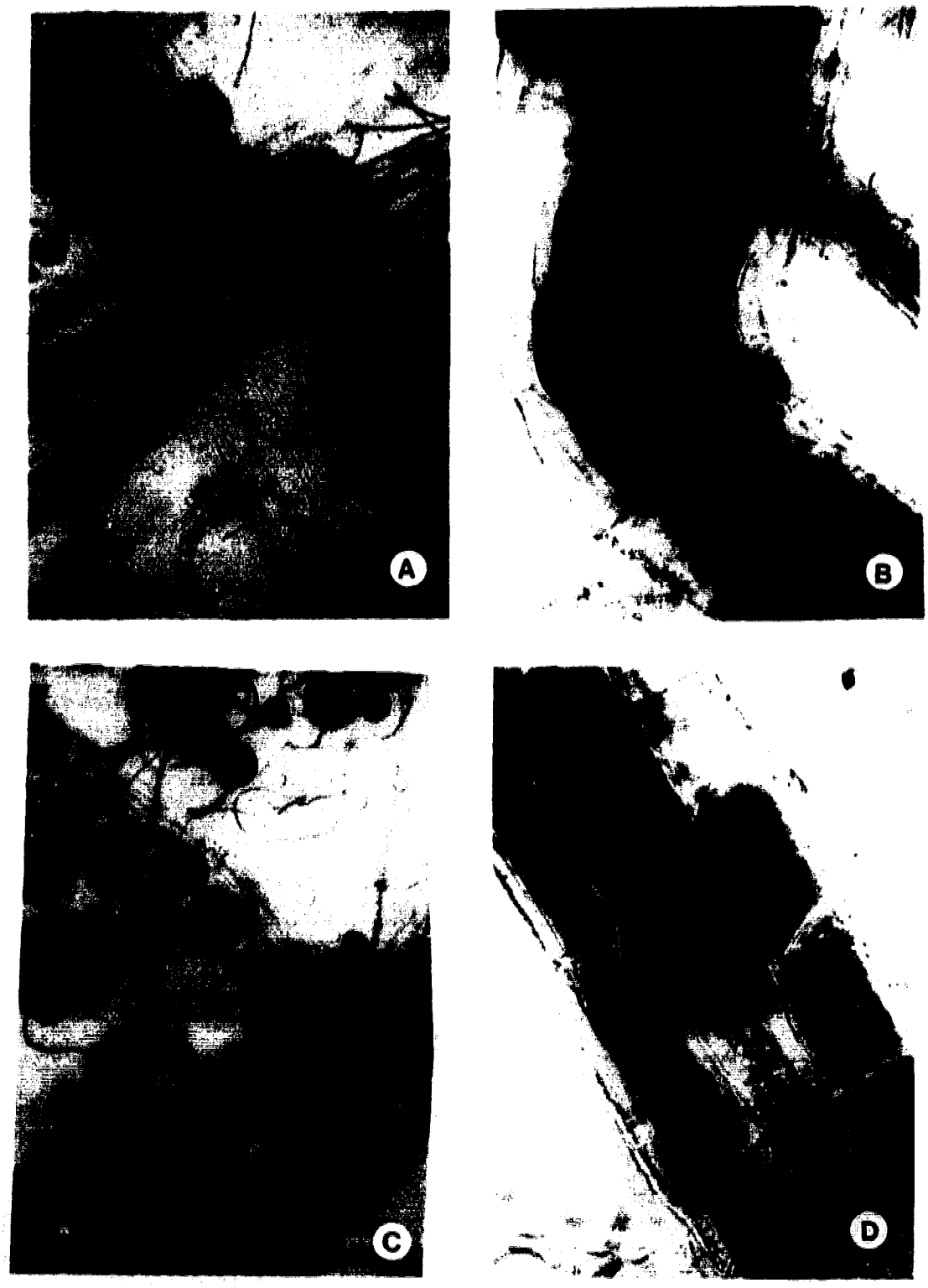
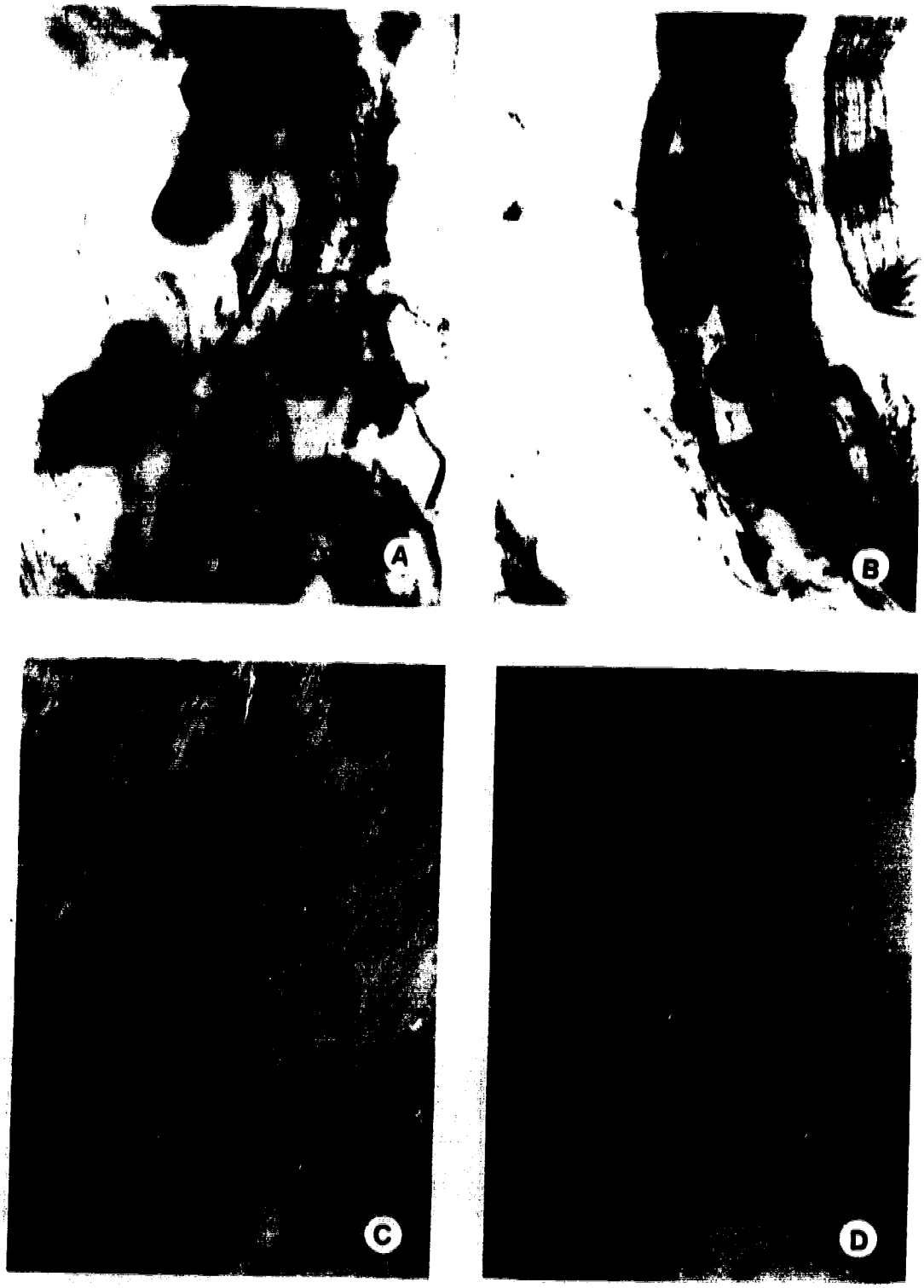

A
B
C
D

Figure 10. Structures formed by *Glomus intraradices* in roots of green foxtail as seen under the light microscope. Note differences in pattern of formation of structures.

A. Spores in a green foxtail root treated with 0.28 kg ai/ha diclofop-methyl. X 115.
B. Spores, arbuscules, and vesicles in green foxtail root treated with 0.28 kg ai/ha diclofop-methyl. X 115.
C. Large number of spores outside the root of green foxtail plants treated with 0.37 kg ai/ha diclofop-methyl. X 115.
D. Arbuscules and vesicles inside the root cortex of green foxtail plants treated with 0.37 kg ai /ha diclofop-methyl. X 396.


A
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The low VAM root colonization in the first run of the experiment initially was attributed to the use of a VAM inoculum culture that was infected with fungal pathogens such as *Pythium* and *Rhizoctonia*. These pathogens are believed to compete for specific niches within the root cortex. Such competition is critical, especially during the first two weeks of plant growth. The pathogens germinate quickly, forming infective pegs and using them to penetrate the root cortex. Once inside the root cortex, they multiply rapidly and form other fungal structures, thus filling most of the available space in it and leaving little space for VAM fungi whose spores germinate slowly and form infective pegs much later (Menge *et al.*, 1990). However, the use of purer VAM inoculum in the second and the third run of the experiment did not dramatically increase the level of root colonization (Tables 1, 2, 3, 4).

VAM fungal infection in control plants (no herbicide applied) did not significantly influence the growth and shoot dry weight of wild oats or green foxtail plants (Table 5, Figures 11, 12, 13, 14).

When wild oats were treated with atrazine, injury symptoms occurred 7 days after herbicide application in both VAM-infected and non-infected plants. The injury symptoms were stunted growth, chlorosis, necrosis, and partial wilting of the plants. These symptoms were most pronounced at the leaf tips and margins (Figure 15). At the low herbicide dose (0.3 kg/ha), some plants recovered from injury but at the higher rate (0.5 kg/ha), some plants died before the end of the experiment. In green foxtail, the symptoms occurred 6 days after atrazine application in both VAM-infected and non-infected plants. The injury symptoms of stunted growth,

Table 5. Shoot dry weights of wild oats and green foxtail plants with or without VAM infection, five weeks after soil treatment with atrazine or diclofop-methyl. Data are means of three experimental runs and are expressed as percentage of control plant dry weight.

	Atrazine			Diclofop-methyl		
	Herbicide dose (kg ai/ha)	VAM	Shoot DW (% of control)	Herbicide dose (kg ai/ha)	VAM	Shoot DW (% of control)
Wild oats	0	-	100	0	-	100
	0	+	102	0	+	109
	0.3	-	29	0.28	-	64
	0.3	+	34	0.28	+	68
	0.5	-	18	0.37	-	57
	0.5	+	18	0.37	+	63
Green foxtail	0	-	100	0	-	100
	0	+	105	0	+	109
	1	-	39	0.28	-	44
	1	+	33	0.28	+	37
	1.5	-	26	0.37	-	32
	1.5	+	22	0.37	+	27

Standard errors ranged from 2 to 25% for wild oats and 2 to 22% for green foxtail.

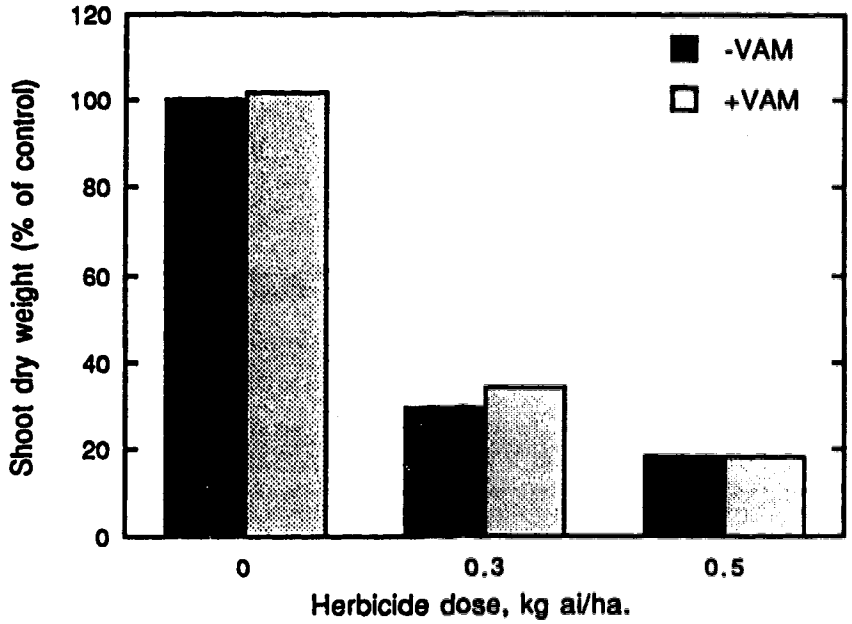

Figure 11. Shoot dry weight of wild oats, five weeks after atrazine application to the soil surface. Means of three experiments. Standard errors ranged from 2 to 25.

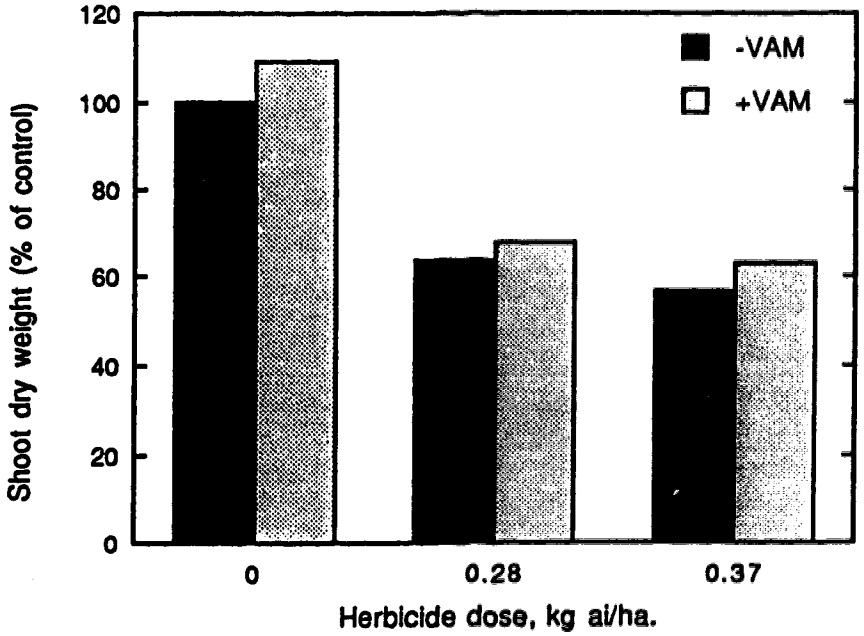

Figure 12. Shoot dry weight of wild oats, five weeks after diclofop-methyl application to the soil surface. Means of three experiments. Standard errors ranged from 2 to 25.

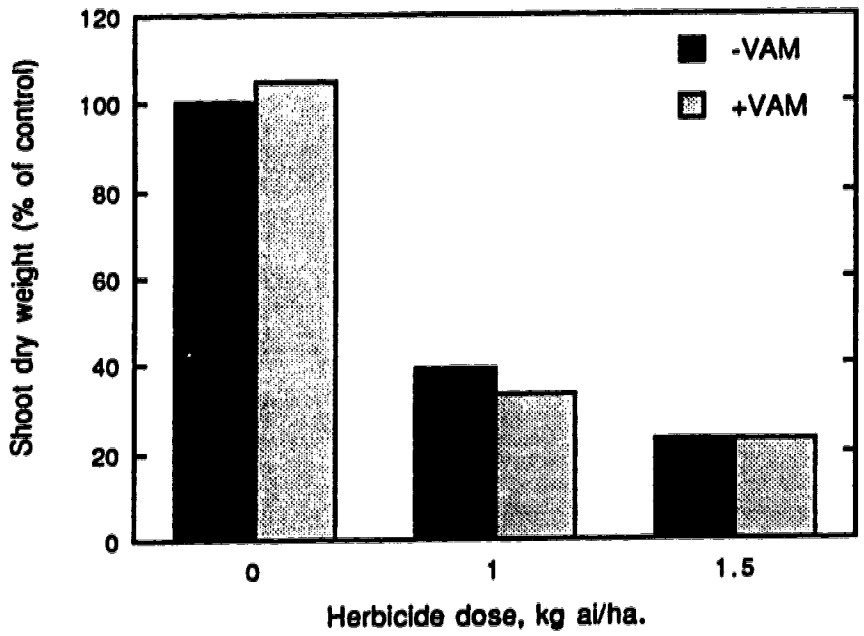

Figure 13. Shoot dry weight of green foxtail, five weeks after atrazine application to the soil surface. Means of three experiments. Standard errors ranged from 2 to 22.

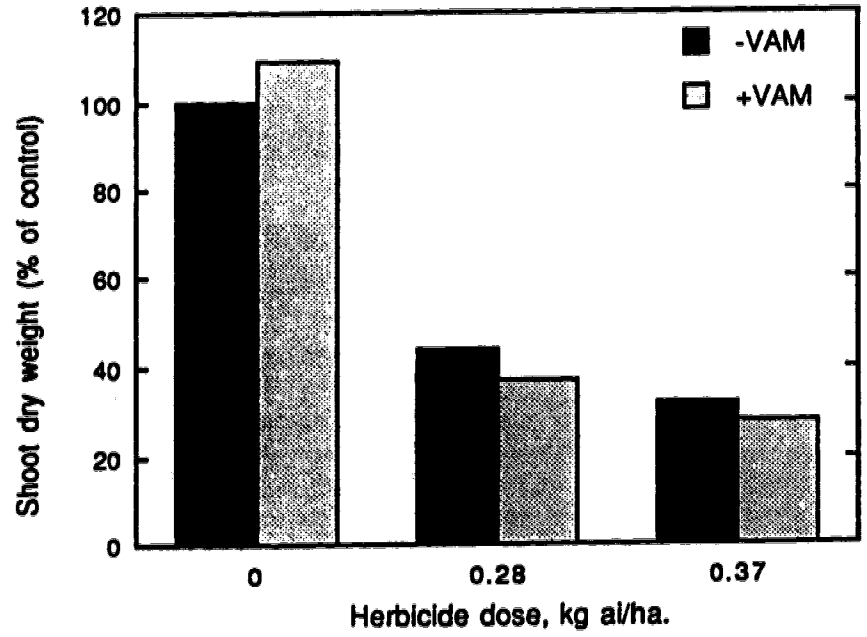

Figure 14. Shoot dry weight of green foxtail, five weeks after diclofop-methyl application to the soil surface. Means of three experiments. Standard errors ranged from 2 to 22.

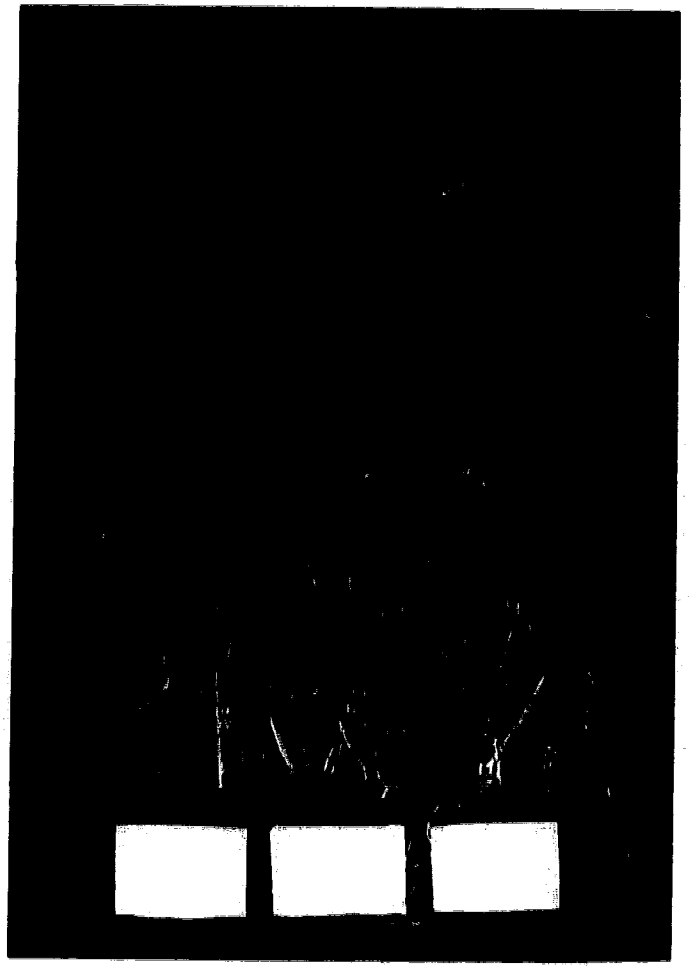

Figure 15. Stunted growth, chlorosis, necrosis, and partial wilting of leaves of wild oats. Left to right: control (no VAM and no herbicide); no VAM, atrazine 0.5 kg/ha; VAM-infected, atrazine 0.5 kg/ha.

chlorosis, browning, necrosis, and wilting initially also were most pronounced at the leaf tips and margins. They later spread to cover most of the leaf (Figure 16). Plant injury was more severe after herbicide treatment at 1.5 kg/ha.

When the wild oat plants were treated with diclofop-methyl, injury symptoms occurred 8 days after herbicide application in the first and the third run of the experiment for both VAM-infected and non-infected plants. In the second run, the appearance of the symptoms was delayed by about 6 days, perhaps as a result of excess water applied by an automatic watering system that leached out some of the herbicide because the soil used in the plastic pots was very sandy. Stunted growth, and chlorosis of old and new leaves, occurred in both VAM-infected and non-infected plants (Figure 17). In green foxtail, injury symptoms of stunted growth, chlorosis, and browning of leaves (Figure 16) occurred 4 days after herbicide treatment in the first and the second run of the experiment but after 8 days in the third run. Herbicide injury was more severe in plants treated at the 3-leaf stage, with little shoot dry weight (Figure 14). These symptoms occurred both in VAM-infected and non-infected plants.

In neither weed species did VAM infection affect the growth or the occurrence of injury symptoms. These symptoms occurred at similar times in VAM-infected and non-infected plants. The changes in shoot dry weight were correlated more to the herbicide dose rate than to VAM infection (Table 5, Figures 11, 12, 13, 14). Thus, these findings indicate that VAM infection did not influence plant response to herbicide uptake.

Figure 16. Stunted growth, chlorosis, browning, necrosis, and partial wilting of leaves of green foxtail.

A. Left to right: control (no VAM and no herbicide); no VAM, atrazine 1.5 kg/ha; VAM-infected, atrazine 1.5 kg/ha.

B. Left to right: control (no VAM and no herbicide); VAM-infected, atrazine 1.5 kg/ha.

C. Left to right: VAM-infected, diclofop 0.37 kg/ha; control (no VAM and no herbicide).

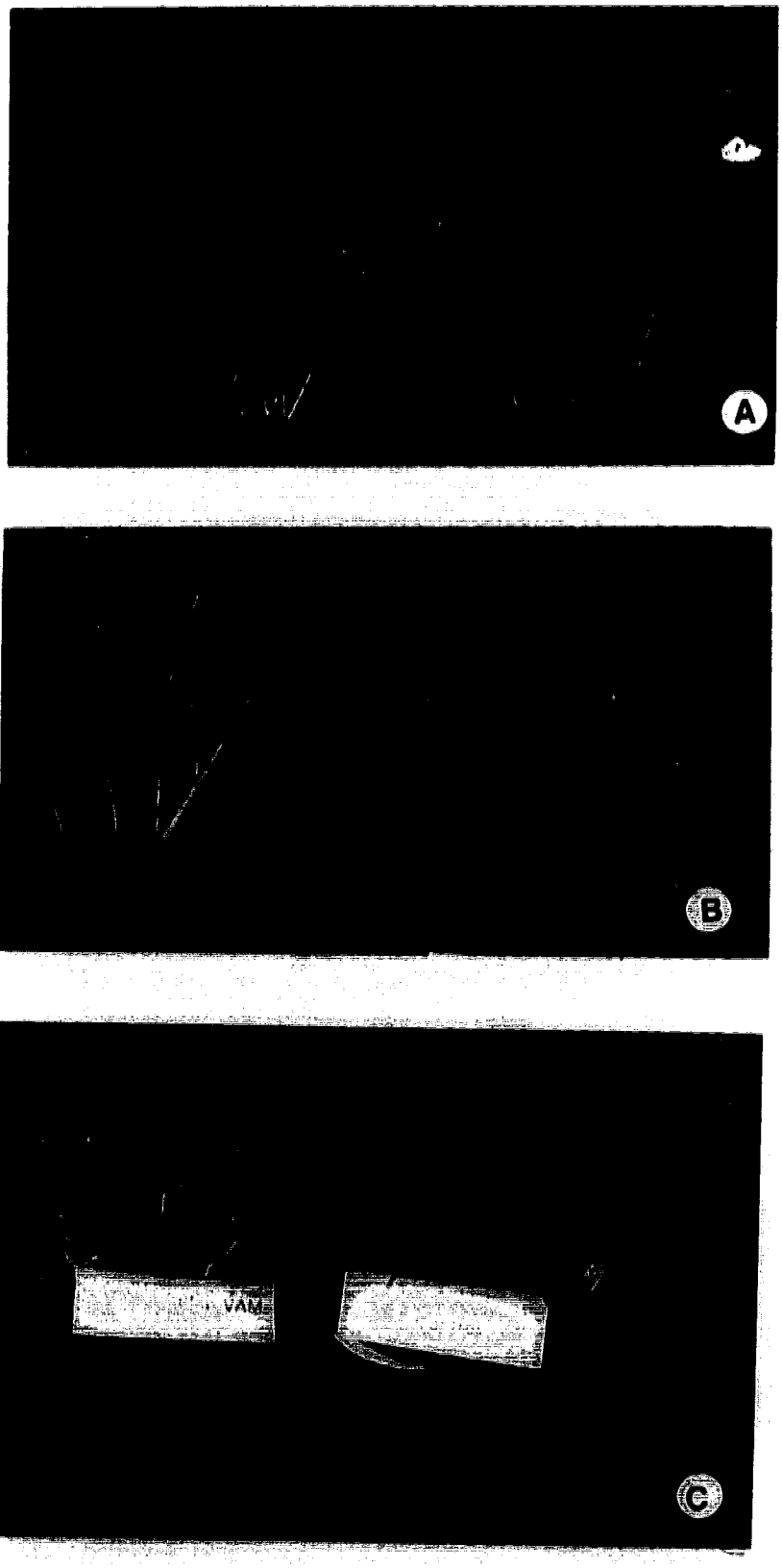
A
B
VAM
C

Figure 17. Stunted growth, chlorosis, necrosis, and wilting of old and new leaves of wild oats treated with diclofop-methyl.
Left to right: control (no VAM and no herbicide); VAM-infected, diclofop-methyl 0.37 kg/ha.

Wild oats and green foxtail plants produced extensive root systems with many root hairs. It has been observed that plants with extensive root systems and many root hairs, that can exploit a large soil volume, generally benefit less from mycorrhizal infection than plants with fairly coarse relatively hairless roots that exploit much less soil (Hayman, 1983; Howeler *et al.*, 1987; Mosse, 1987). The large root mass produced by these plants could, therefore, have masked the effects of VAM.

These results offered no consistent indication that VAM fungal infection might result in more rapid or extensive injury to wild oat and green foxtail plants from soil-applied herbicides.

4. 2. Uptake of ^{14}C-atrazine by VAM-infected Roots of White Clover and Green Foxtail

4. 2. 1 Plant Growth

VAM-infected plants were grown in a growth cabinet in specially constructed petri dishes. Uptake by root systems with or without VAM was compared to uptake solely via VAM hyphal systems by controlling access to ^{14}C-atrazine-treated soils.

Seed germination was uneven, especially in the first experiment, due to low day/night temperatures of 22 C/20 C used initially in the growth cabinet. This necessitated trimming/pruning of the plants to bring them to uniform size at the time of herbicide treatment and harvesting. In particular, the main stem of green foxtail was cut down several weeks before atrazine treatment to stimulate lateral growth and production of new shoots. This weed is

an annual that flowers early, initiates seed set, and senesces. Hence, the cutting of the main stem was done to extend the duration of vegetative growth until the end of the experimental period. Despite this trimming, the plants produced variable shoot and root weights, especially white clover, and mainly in the first experiment.

White clover plants produced a sizeable root system, while green foxtail produced a massive amount of roots, with most of the root systems in the lower compartment of the petri dishes.

4. 2. 2. VAM Infection

Both the white clover and green foxtail plant roots were readily infected by VAM. The level of root colonization by the mycorrhizal fungi for white clover plants was 48% in the first experiment and 59% in the second experiment, while it was 62% and 69% for green foxtail. These infection levels are in the same range as those of the pot grown plants described earlier. Roots in both soil compartments of the treatment vessels contained structures characteristic of VAM infection. Many spores, arbuscules, vesicles, intra- and extamatrical hyphae were observed within the root cortex region of both white clover plants and green foxtail plants (Figure 18). VAM fungi, once inside the root cortex region of white clover, formed an extensive pattern of infection of well developed arbuscules and vesicles. Many fungal structures, especially young spores, were formed on the root surface of green foxtail (Figure 18) within two months of plant infection, indicating that these plants can be used as hosts for quick multiplication of VAM inoculum.

Figure 18. Structures formed by *Glomus intraradices* in roots of white clover and green foxtail plants, as seen under light microscope. Note differences in pattern of formation of structures.

A. Extensive mycelium and large number of spores and vesicles in roots of white clover plants used in ^{14}C-atrazine uptake experiment. X 115.

B. Arbuscules inside the root cortex of white clover plants used in ^{14}C-atrazine uptake experiments. X 115.

C. Large number of spores outside the roots of green foxtail plants used in ^{14}C-atrazine uptake experiments. X 115.

D. Arbuscules and vesicles inside the root cortex of green foxtail plants in used in ^{14}C-atrazine uptake experiments. X 115.


A
B
C
D

No vesicles or arbuscular structures were observed in the roots of uninoculated plants.

4. 2. 3. White Clover

Radioactivity determinations were made on plants harvested 72 and 144 h after applying ^{14}C-atrazine solution to the lower compartment of the specially constructed petri dishes. Uptake of ^{14}C-atrazine via the roots in the lower compartment after 72 and 144 h accounted for 9% and 16-19%, respectively, of the amount of radioactivity supplied (Table 6). By contrast, uptake solely via the hyphal system was only 0.1% of the radioactivity supplied, or about 1.5% of the amount taken up directly by roots.

After uptake by bare roots, or by the root/VAM complex, 80-90% of the ^{14}C-atrazine was translocated to plant shoots (Table 6). When uptake from soil occurred only via the hyphal system, a smaller proportion of the radioactivity taken up (66-67%) was transferred to shoots and a larger proportion remained in the roots. Nevertheless, since total uptake was much less in this instance, the absolute amount of radioactivity retained in the roots was small. The amount of ^{14}C-atrazine in the roots varied from 8 to 34% (Table 6). Between 0.1 and 19% of the atrazine solution supplied to the plant was accounted for in the plant tissue and the rest remained in the soil, in solution or adsorbed as no leaching of the herbicide was observed.

The hyphal systems were able to take up ^{14}C-atrazine and transfer it to white clover plants. However, the amount taken in through this pathway was small, amounting to 1.5% of the amount taken in by intact root systems (Table 6). The fraction taken in by

Table 6. Dry weights of roots, leaves, petioles, and stems, and radioactivity of white clover plants treated with ^{14}C-atrazine. Plants were harvested 72 h and 144 h after the herbicide treatment and dried at 65C for 24 h to constant weight. Plant tissue samples were analysed for radioactivity, and total uptake per plant was determined. Data are means of two four-replicate experiments.

Plant part	Uptake period	No VAM infection		VAM-infected: Roots in both compartments		VAM-infected: Roots in top compartment only	
		DW (g)	DPM ('000)	DW (g)	DPM ('000)	DW (g)	DPM ('000)
Leaves	72 h	0.19	149.6	0.31	116.4	0.30	0.9
Petioles + stems		0.17	31.3	0.31	56.6	0.24	0.8
Shoot total		0.36	180.9	0.62	173.0	0.54	1.7
Roots		0.48	37.3	0.55	38.1	0.61	0.9
Plant total		0.84	218.2	1.17	211.1	1.15	2.6
% of ^{14}C in shoots			83		82		66
% of ^{14}C in roots			17		18		34
% of total applied			9		9		0.1
Leaves	144 h	0.31	311.5	0.43	390.0	0.44	1.4
Petioles + stems		0.30	41.9	0.40	56.2	0.33	0.5
Shoot total		0.61	353.4	0.83	446.2	0.77	1.9
Roots		0.48	40.1	0.55	39.1	0.53	0.9
Plant total		1.09	393.5	1.38	485.3	1.30	2.8
% of ^{14}C in shoots			90		92		67
% of ^{14}C in roots			10		8		33
% of total applied			16		19		0.1

Standard errors for dry weight data means were 0.05 to 0.17; for radioactivity they were 0.25 to 18 ('000).

hyphal systems did not differ for the two harvesting periods (Table 6, Figure 19).

VAM-infected plants did not take up significantly more ^{14}C-atrazine (statistically) than non-infected plants (Table 6, Figure 19). This was attributed to very low levels of uptake by the hyphal network systems. These levels were considered too low to be evident in the root system uptake treatments as a direct effect of VAM on enhanced root system contact with ^{14}C-atrazine-treated soils via the attached hyphal network.

Total dry weights of the plants with roots in both lower and upper compartments of the petri dishes varied considerably (Table 6), despite trimming of the plants and controlling the growth environment in the cabinet.

4. 2. 4. Green Foxtail

Uptake of ^{14}C-atrazine by green foxtail plants was lower than by white clover. At 72 h, uptake accounted for 6% of the dose applied, and after 144 h it accounted for 9-11% (Table 7). Uptake solely via the hyphal system was 0.2% of the dose applied or 2-3% of the amount taken up directly by bare roots or the root/VAM complex. Between 0.2 and 11% of the ^{14}C-atrazine applied was accounted for in the plant tissue. The total plant uptake was low as compared to white clover plants, mainly due to less leaf tissue produced by green foxtail, thus exposing less surface area for transpiration, movement and accumulation of herbicide in the leaf tissues.

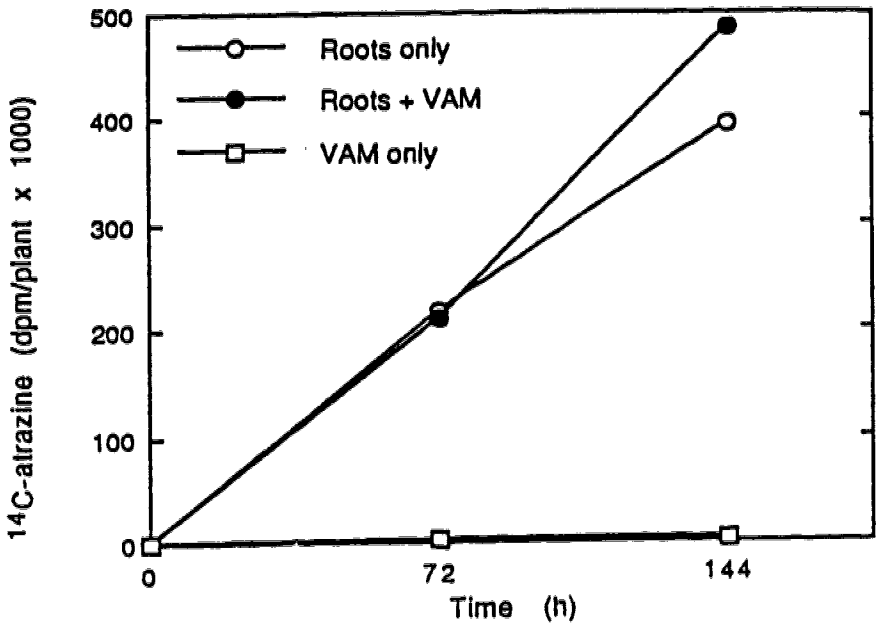

Figure 19. ^{14}C-atrazine uptake by white clover 72 h and 144 h after the herbicide treatment. Data are means of two four-replicate experiments. Standard errors ranged from 0.25 to 18 ('000).

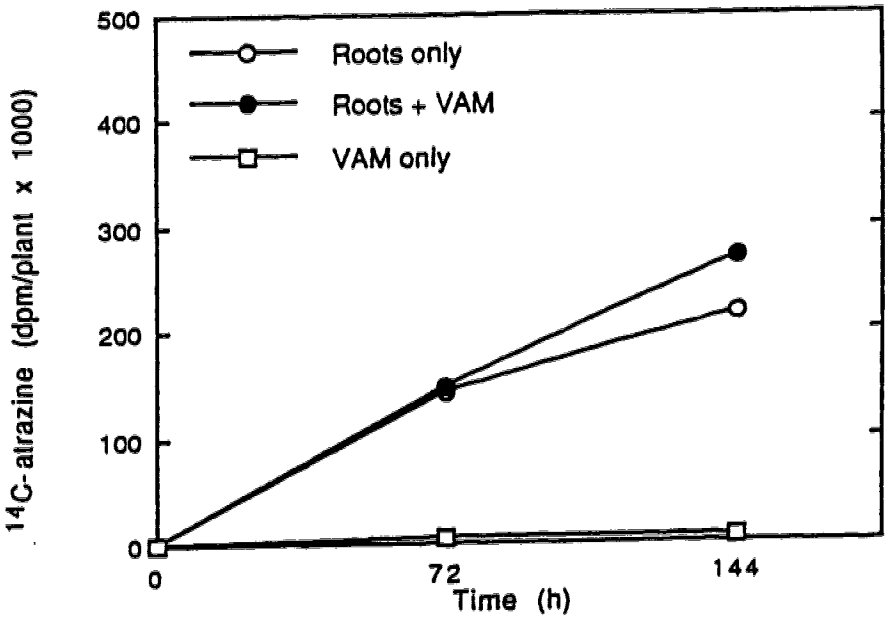

Figure 20. ^{14}C-atrazine uptake by green foxtail 72 h and 144 h after the herbicide treatment. Data are means of two four-replicate experiments. Standard errors ranged from 0.20 to 23 ('000).

Table 7. Dry weights of roots, leaves, petioles, and stems, and radioactivity of green foxtail plants treated with ^{14}C-atrazine. Plants were harvested 72 h and 144 h after the herbicide treatment and dried at 65C for 24 h to constant weight. Plant tissue samples were analysed for radioactivity, and total uptake per plant was determined. Data are means of two four-replicate experiments.

Plant part	Uptake period	No VAM infection		VAM-infected: Roots in both compartments		VAM-infected: Roots in top compartment only	
		DW (g)	DPM ('000)	DW (g)	DPM ('000)	DW (g)	DPM ('000)
Leaves	72 h	0.16	66.5	0.17	67.2	0.14	1.8
Petioles + stems		0.63	36.5	0.63	43.2	0.53	1.9
Shoot total		0.79	103.0	0.80	110.4	0.67	3.7
Roots		0.99	39.7	0.89	36.0	0.59	1.6
Plant total		1.78	142.7	1.69	146.4	1.26	5.3
% of ^{14}C in shoots			73		75		70
% of ^{14}C in roots			27		25		30
% of total applied			6		6		0.2
Leaves	144 h	0.17	115.0	0.17	134.0	0.14	2.5
Petioles + stems		0.64	59.9	0.72	87.2	0.48	1.6
Shoot total		0.81	174.9	0.89	221.2	0.62	4.1
Roots		0.90	41.8	0.95	50.2	0.51	1.6
Plant total		1.71	216.7	1.84	271.4	1.13	5.7
% of ^{14}C in shoots			81		81		72
% of ^{14}C in roots			19		19		28
% of total applied			9		11		0.2

Standard errors for dry weight data means were 0.06 to 0.15; for radioactivity they were 0.20 to 23 ('000).

After direct uptake by roots, 73 to 81% of the ^{14}C-atrazine was translocated to plant shoots (Table 7). When uptake from soil occurred via the hyphal system, a slightly smaller proportion of the radioactivity taken up (70-72%) was transferred to shoots and a larger proportion remained in the roots. Nevertheless, since total uptake was much less in this instance, the absolute amount of radioactivity retained in the roots was small. The amount in the root tissues varied from 19 to 30% of the total in the plant (Table 7).

The hyphal systems were able to take up ^{14}C-atrazine and transfer it to green foxtail plants. However, as with the white clover plants, the amount taken up through this pathway was small, amounting to 2-3% of the amount taken up by the complete root systems. The fraction taken in by the hyphal systems did not differ for the two harvesting periods (Table 7, Figure 20).

There was no significant difference in uptake of ^{14}C-atrazine between plants with VAM-infected and uninfected roots (Table 7, Figure 20). The lack of statistical significance in these comparisons was attributed to the very low levels of uptake by hyphal network systems.

The total dry weights of the plants varied considerably, as they did in white clover.

These results agree with observations of other researchers. Khan *et al.* (1992) showed that VAM inoculation enhanced the uptake and transfer of ^{14}C-atrazine through a hyphal pathway to corn plants. Van Kessel *et al.* (1985) and Hamel *et al.* (1991) showed that VAM enhanced the transfer of ^{15}N from soybean to maize. Barea *et al.* (1989) and Haystead *et al.* (1988) showed that ^{15}N applied to white

clover was actively transferred via a hyphal pathway to companion rye grass. These authors also concluded that the differences in uptake between VAM-infected and non-infected root systems were not statistically significant because the contribution via the hyphal network systems was very small.

5. Summary and Conclusions

It is evident from the greenhouse and growth cabinet experiments that VAM fungi formed symbiotic associations with the test weeds, wild oats, green foxtail, and white clover plants. The VAM fungi infected and colonized considerable portions of the root systems of the various host plants, with the formation of well developed fungal structures such as spores, hyphae, arbuscules, and vesicles. The level of root colonization varied among the test species and ranged from 26 to 52% for wild oats, 48 to 69% for white clover, and 45 to 70% for green foxtail plants.

The extent, susceptibility, and pattern of infection of roots differed among the test species. The differences suggested that VAM fungi have certain host preferences or that the host influences their ability to colonize plant roots. Other researchers have also observed significant differences in extent, susceptibility, and pattern of infection among cultivars of various plant species (Boyetchko, 1991; Hetrick *et al.*, 1985; Azcon and Ocampo, 1981; Mercy *et al.*, 1990).

Treatment with the herbicides atrazine and diclofop on wild oats and green foxtail plants, did not affect the level of root colonization by VAM fungi. These results suggested that the herbicide treatment had no observable effect on VAM fungal growth and development. Treatments with herbicides at low or recommended field rates have been reported to have no effect on fungal growth and development (Nemec and Tucker, 1983; Smith et al., 1981). Trappe *et al.* (1984), for example, observed: "with few exceptions, the herbicide concentrations necessary to affect VAM

fungal growth significantly were considerably higher than would be expected to occur in soil treated with test herbicides at recommended application rates".

VAM inoculation did not result in increases in plant growth. It was visually observed that VAM-infected plants were more vigorous than the non-infected ones. This, however, was not reflected in dry matter yield data. There have been different reports regarding the significance of VAM in plant growth. Increases in plant growth and yield of barley have been observed (Mosse *et al.*, 1981; Jensen, 1982). Khan (1972; 1974) reached similar conclusions with maize and wheat. Increases in plant growth and yield in most cases have been associated with increased P uptake by the fungal hyphae. Other researchers observed reduced plant growth. Simpson and Daft ((1990) reported a reducton of growth of maize and sorghum infected with VAM. These reductions have been attributed to competition for photosynthates between the VAM fungi and the host plant and indicate that not all VAM fungi are beneficial to the host.

VAM fungal infection did not contribute to more rapid or extensive injury to wild oats or green foxtail growing in soils treated with atrazine or diclofop. There were no significant differences in shoot dry weights of VAM-infected herbicide-treated and uninfected herbicide-treated plants. The hyphal contribution to total uptake was (1-3%) in VAM-infected plants, too small to show significant differences with non-infected plants.

Hence, these results offered no indication that VAM fungal inoculation could result in more rapid or extensive injury to wild oats and green foxtail plants from soil-applied herbicides.

The ^{14}C-atrazine uptake results showed that there was more radioactivity in white clover than in green foxtail, with more than 80% contained in the shoot tissues as compared to 70% in green foxtail plants. Nelson and Khan (1992). observed similar values in maize.

There was no significant difference in uptake of ^{14}C-atrazine after 72 h or 144 h between VAM-infected and uninfected plants. However, when the roots were confined to the upper compartment of specially constructed petri dishes so that only hyphae made contact with the herbicide-treated soil in the lower compartment, the hyphae supplied the plant with small quantities of the herbicide, amounting to 1-3% of that supplied by a complete root system.

Similarly, Abbott *et al.* (1992) observed that external hyphae of VAM could transport ^{32}P to *Trifolium substerraneum* L. They concluded that the efficiency of P uptake by VAM fungi was strongly affected by spatial distribution of hyphae in the soil and possibly also by unit length of hyphae. Kothari *et al.* (1991) demonstrated that VAM fungal hyphae increased maize plant acquisition of P and Zn. Marshner *et al.* (1991) observed the uptake and translocation of P by the external hyphae of VAM to white clover plants. Beat Frey and Hannes Schuepp (1993) reported the acquisition of ^{15}N by external hyphae of VAM fungus to infected maize plants.

The VAM hyphae, therefore, can contribute to herbicide uptake by plants with VAM-infected roots but their contributon is small. Whereas there are indications that VAM fungi can increase plant growth and yield through the transport of mineral nutrients,

especially P, along the external hyphae into plant roots, the increased uptake and efficacy of soil-applied herbicides through similar pathways may not be of much practical significance. These results showed that the hyphal uptake contribution in VAM-infected weed plants was too small to result in any significant differences with non-infected plants. The use of VAM fungi in weed control programmes, therefore, may, be of little practical consequence.

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Appendix 1

Table A1. Dry matter yield of wild oats treated with atrazine (Experiment 1).

Herbicide treatment (Kg ai/ha)	VAM inoculation, rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	8.76	100	0.28
0.3	0	2.72	31	0.36
0.5	0	1.72	20	0.32
0	6000	8.54	97	0.74
0.3	6000	3.06	35	0.30
0.5	6000	1.46	17	0.16

Table A2. Dry matter yield of wild oats treated with atrazine (experiment 2)

Herbicide treatment (kg ai/ha)	VAM inoculation, rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	14.61	100	0.87
0.3	0	4.91	34	0.53
0.5	0	2.66	18	0.69
0	5500	15.28	104	0.78
0.3	5500	5.20	36	0.71
0.5	5500	2.28	16	0.45

Table A3. Dry matter yield of wild oats treated with atrazine (experiment 3).

Herbicide treatment, (kg ai/ha)	VAM inoculation, rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	10.75	100	0.21
0.3	0	2.32	22	0.11
0.5	0	1.70	16	0.26
0	6900	11.50	106	0.34
0.3	6900	3.48	32	0.26
0.5	6900	2.13	20	0.12

Table A4. Dry matter yield of wild oats treated with diclofop-methyl (experiment 1).

Herbicide treatment (kg ai/ha)	VAM inoculation rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	9.00	100	1.00
0.28	0	5.35	59	1.20
0.37	0	3.98	44	0.27
0	6000	9.63	107	0.38
0.28	6000	5.52	61	1.00
0.37	6000	5.09	57	0.25

Table A5. Dry matter yield of wild oats treated with diclofop-methyl (experiment 2).

Herbicide treatment (kg ai/ha)	VAM inoculation rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	13.57	100	0.93
0.28	0	8.10	60	0.94
0.37	0	7.80	57	0.93
0	5500	13.43	99	1.50
0.28	5500	8.92	66	0.73
0.37	5500	8.33	61	0.68

Table A6. Dry matter yield of wild oats treated with diclofop-methyl (experiment 3)

Herbicide treatment (kg ai/ha)	VAM inoculation rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	9.09	100	1.02
0.28	0	6.53	72	0.86
0.37	0	6.42	71	0.40
0	6900	11.00	121	0.43
0.28	6900	7.11	78	0.55
0.37	6900	6.44	71	0.47

Table A7. Dry matter yield of green foxtail treated with atrazine (experiment 1).

Herbicide treatment (kg ai/ha)	VAM inoculation rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	3.21	100	0.30
1.0	0	0.60	19	0.09
1.5	0	0.29	9	0.05
0	6000	2.72	84	0.08
1.0	6000	0.47	15	0.04
1.5	6000	0.30	9	0.01

Table A8. Dry matter yield of green foxtail treated with atrazine (experiment 2).

Herbicide treatment (kg ai/ha)	VAM inoculation rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	8.16	100	2.43
1.0	0	5.96	73	0.65
1.5	0	3.29	40	0.60
0	5500	10.09	122	2.20
1.0	5500	5.15	63	0.93
1.5	5500	3.18	39	0.62

Table A9. Dry matter yield of green foxtail treated with atrazine (experiment 3).

Herbicide treatment (kg ai/ha)	VAM inoculation rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	10.62	100	0.37
1.0	0	2.67	25	0.18
1.5	0	2.00	19	0.14
0	6900	12.58	109	0.5
1.0	6900	2.18	20	0.08
1.5	6900	1.82	17	0.11

Table A10. Dry matter yield of green foxtail treated with diclofop-methyl (experiment 1).

Herbicide treatment (kg ai/ha)	VAM inoculation rate (spores/100 g soil)	Mean DM yield	% of control	SE
0	0	1.80	100	0.13
0.28	0	0.39	22	0.04
0.37	0	0.40	22	0.03
0	6000	1.60	89	0.07
0.28	6000	0.34	19	0.01
0.37	6000	0.26	14	0.05