EFFECTIVENESS OF ARBUSCULAR MYCORRHIZAL FUNGI IN INCREASING *ACACIA KOA* AND *LEUCAENA LEUCOCEPHALA* TOLERANCE TO ALUMINUM AND MANGANESE TOXICITIES

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CHAPTER 1: INTRODUCTION

1.1. Problem statement

Soil acidity is one of the most important limiting factors for crop production worldwide (John et al., 2005; Uexkull and Muter, 1995). Acidic soils are characterized by a relatively high concentration of H^+ and a pH value of 5.5 or lower. However, depending on plant species and cultivars, low pH itself is often may not be the cause restricting plant growth on these soils. The main constraints in acid soil are usually toxic levels of aluminum (Al), manganese (Mn) and deficiency of nutrients such as calcium (Ca), magnesium (Mg), phosphorus (P) and molybdenum (Mo) (Kochian et al., 2003).

Acid soils differ significantly due to diverse factors in soil formation, especially differences in climate, parent material and vegetation type. Soils that have developed under humid temperate, humid tropical and subtropical climatic conditions are usually acidic in their natural state (Habte, 1995). Many tropical soils such as Oxisols and Ultisols, that are extremely weathered, fall into this category. They have a high amount of exchangeable Al or Mn due to the advanced state of soil weathering processes.

Aluminum and Mn are highly soluble at a low pH and may be detrimental for plant growth at a relatively low concentration. In addition to Al and Mn toxicities, soil acidity conditions have several adverse effects on soil nutrient availability. For instance, Al reduces inorganic P (Pi) availability by forming a complex Al-Pi that precipitates in the rhizosphere, restricts P uptake by the plant (Cumming et al., 1986). In addition, Al also interacts with Ca and Mg and limits their uptake and translocation in the plant.

Moreover, high moisture in acid soil often leads to the leaching of cationic elements such as Ca, Mg, and K from the root growth zone.

The common management practices used to overcome the adverse effects of acidic soils are liming or the introduction of acid tolerant species. However, liming may be costly and its effect is not long term. Fortunately, the use of arbuscular mycorrhizal fungi (AMF) combined with acid tolerant cultivars might alleviate the detrimental effect of soil acidity. However, the effectiveness of AMF to protect *Acacia koa* and *Leucaena leucocephala* against Al and Mn toxicities and to improve growth on acid soil has not been studied.

1.2. Justification

The competition with third-world countries had caused a dramatic decline of the sugarcane industry in Hawaii. As a result, many sugarcane plantations were left uncultivated. In the last two decades, reforestation of some of these lands with *Acacia koa is* of potential interest due to the high cultural and economical value of this fast growing nitrogen fixing tree. Koa is prized for its high quality wood (Whitesell, 1964). According to a forest survey conduced by Hawaiian Agriculture Research Center (HARC) the area covered by *A. koa* forest is decreasing. Reforestation of fallowed sugarcane lands may be an environmentally sound solution for rebuilding the koa population. Some of these lands may consist of highly weathered Oxisols and Ultisols that are characterized by a low pH and associated toxicities of Al^{3+} and Mn^{2+}, coupled

with deficiencies in P, Ca, and Mo. These conditions often limit plant growth (Marschner, 1991; Sanchez, 1992) of leguminous species such as *A. koa* and *L. leucocephala*. Determination of the effectiveness of acid tolerant AMF in increasing *A. koa* and *L. leucocephala* growth under soil Al and/or Mn toxic conditions will enable us to evaluate to which extent the use of AMF can be an appropriate substitute to liming.

The overall goal of the current study is to assess whether *A. koa* and *L. leucocephala* can grow on acid soil with associated Al and Mn toxicity by the use of acid tolerant AMF.

1.3. Rationale

The effectiveness of AMF to protect *A. koa* and *L. leucocephala* against Al and Mn toxicities may depend on several factors such as the level of tolerance of both the AMF and host plant to soil acidity, the host-endophyte compatibility, the capacity of the AMF on improving the host nutrient status, and its ability to reduce the uptake of toxic elements such as Al and Mn.

1.4. Hypotheses

- The initial Al and Mn concentrations in Al-toxic Leilehua and Mn-toxic Wahiawa soils are toxic to *Acacia koa.*
- Inoculation of *A. koa* with AMF improves the growth of *A. koa* under acidic conditions.
- Arbuscular mycorrhizal fungi effectiveness in alleviating Al and Mn toxicities depends on plants genotype.

- Different AMF isolates have different effectiveness in alleviating the effect of Al and Mn toxicities on the growth of *A. koa*

1.5. Objectives

(i) To determine the levels of Al and Mn that are toxic to *A. koa* and *L. leucocephala.*

(ii) To determine the effectiveness of acid-tolerant isolates of AMF on the growth of *A. koa* and *L. leucocephala* in acid soil containing toxic level of Al and Mn.

(iii) To determine the extent to which the effectiveness of AMF in alleviating the effect of Al and Mn toxicities on *A. koa and L. leucocephala* can depend on plant genotype.

(iv) To determine the effectiveness of two isolates from Leilehua and Wahiawa soils in decreasing the negative effect of Al and Mn toxicities on the growth of *A. koa* in acidic soils.

CHAPTER 2: LITERATURE REVIEW

2.1. Soil Acidity

Soil acidity is a major concern in many regions of the world. Acid soils, (i.e. soils with low pH) limit crop production worldwide. According to Sanchez and Logan (1992), they occupy a major part of the world's arable land. The majority of the world acid soils are located in the tropics and sub-tropic (Kochian, 2004) due to the warm climate and high rainfall in these regions that accelerate weathering. Therefore, soil types such as Oxisols and Ultisols are typically acid in their natural state. According to FAO/UNESCO maps (www.fao.org/agl/agll/wrb/mapindex.stm, 1998), most Oxisols and Ultisols are located in the tropics and sub-tropics. In the USA, where the majority of acid soils are Ultisols, only the state of Hawaii has Oxisols (Hue et al., 1994).

The low pH in an acid soil usually leads to high levels of Al and/or Mn in the soil solution. Consequently, poor plant growth on these soils is associated with Al and/or Mn toxicity. In addition, soil acidity also causes nutrients deficiencies of P, Ca, Mg, or Mo. In order to be available and toxic for the plants, Al must be in the soil solution (Adams, 1984). According to Hue et al. (1994) Al in the soil originates from primary and secondary mineral solids, particularly aluminosilicates such as feldspars, micas, kaolins, smectite, and vermiculites. As a result of weathering under rainfall and warm temperature, silica is leached as $Si(OH)_4$ leaving Al behind in the solid forms of Al oxyhydroxides, such as boehmite and gibbsite.

$$Al_2Si_2O_5(OH)_4 + 5\ H_2O \longleftrightarrow 2Al(OH)_3 + 2Si(OH)_4$$

Kaolinite Gibbsite

Several processes are involved in the control of the solubility of Al in soil. Figure 2.1 describes some of them.

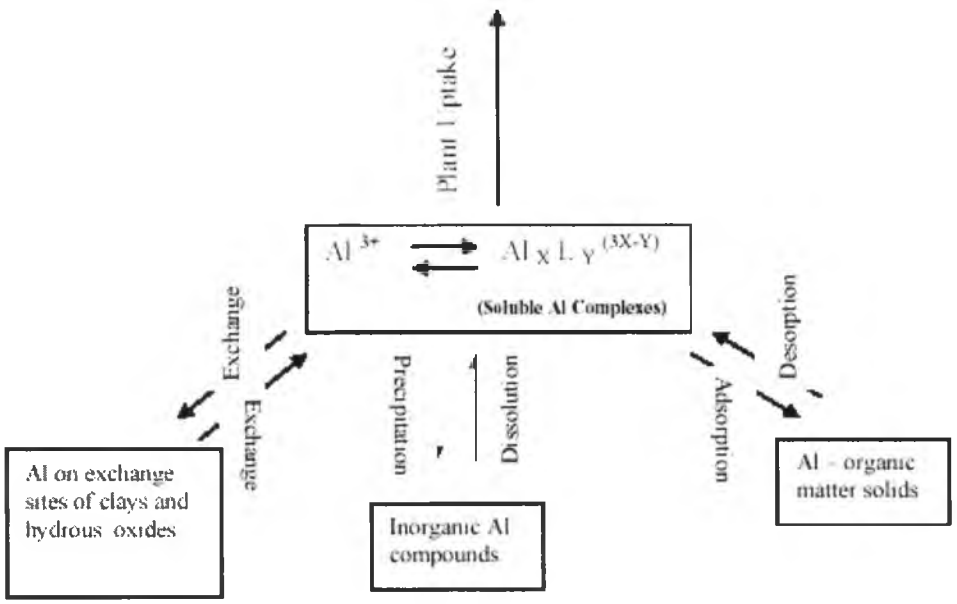

Figure 2.1. Processes that control forms, solubility, and availability of Al in soils. Source: Allen (2006).

Manganese toxic acid soils are less widespread globally compared with Al-toxic ones. However, Mn-toxic acid soils do exist in several locations in the world particularly in Hawaii (Hue et al., 1998). It has been reported by Hue et al. (1998) that a major portion of agricultural land on Oahu, consist of soils with 10 to 14 g kg^{-1} total Mn concentration. Mn-toxic soils are mostly Oxisols of basaltic origin and are located in area of low

elevation (Hue et al., 2001). Manganese in the soil occurs as exchangeable Mn, organic manganese, manganese oxide and component of Ferro–magnesian silicate minerals (Barber, 1995). Soil Mn exists in different oxidative states such Mn^{+2}, Mn^{+3} and Mn^{4+}. But, Manganese is often present in the soil solution as Mn^{2+}. However, this essential nutrient can be toxic to crops when occurring in excess (Marschner, 1995). Moreover, Mn concentrations in soils can be high enough to reduce plant growth before visible toxicity symptoms are observed (Arines et al., 1989). Manganese availability depends essentially on factors such as soil pH, Eh, organic matter and microbial activity (Hue, et al., 2001).

In soil with a high Mn content, toxicity may occur sometimes at a pH as high as 6.5 (Yost, 2006 personal communication), a pH level at which Al is not available in soil solution. This situation may occur when plants acidify their rhizosphere. Although, Mn toxicity could cause damage to plants, numerous studies have shown that its availability appears to be affected by the presence of AMF (Nogueira et al., 2002; Benthlenfalvay, 1989; Kochian et al., 2004).

2.2. The use of AMF

Arbuscular mycorrhizal fungi are the most common underground symbionts (Smith and Read, 1997). They form a beneficial association with roots of most crops and natural occurring plant species. The symbiosis of AMF with plant roots of almost all higher plants species clearly enhance the uptake of diffusion-limited nutrients from the soil, in particular P, Cu and Zn. The nutritional significance of AMF is due to the volume of soil explored by hyphae that act as extension of host plant roots and by increasing their

absorbing surface. Arbuscular mycorrhizal fungi are also known to be involved in providing to the host several benefits such as protection against pathogens and environmental stress (drought, and salinity) (Al-Karaki, 2000). In many tropical soils in which P is generally poorly supplied, AMF could play an important role in improving plant productivity (Habte and Soedarjo, 1996). However, it should be noted that AMF establishment depends on several soil factors, such as soil nutrient levels and soil physical properties such as structure and texture (Nogueira and Cardosso, 2003). Several authors stated that soil acidity and associated toxicities affect the formation and function of some AMF (Foy, 1983; Habte, 1995). Clark and Zeto (1996) found that most of the AMF appear to be adapted to soil pH conditions close to those from which they were isolated. They found that *Glomus* isolated from acid soil effectively increased maize growth on soils ranging in pH from 4.7 to 8. Therefore, some AMF have the ability to be efficient in both acid and alkaline soils whereas, others are more acid or alkaline tolerant.

The ability of plants to grow in acidic conditions may be associated with AMF colonization of roots and their adaptability to low pH (Kolowsky and Boener, 1989). There is some evidence that the adverse effect of acid soil could be ameliorated through the use of acid-adapted AMF endophytes (Habte, 1995). Arbuscular mycorrhizal fungi association specifically, could alleviate Al toxicity (Nogueira et al., 2002; Cuenza et al., 2001; Clark, 1997; Kolowsky and Boener, 1989; Siqueira et al., 1984) or Mn toxicity (Nogueira and Cardoso, 2003). In addition, Mosse (1981) reported that AMF are believed to improve soil aggregation by increasing soil aggregate weight from 0.9 to 2.5 g kg^{-1}, considerably improving soil structure.

These positive effects may be attributed to the fact that AMF constitute an efficient root extension organ involved in uptake and translocation of nutrients with low diffusion rates. The AMF-plant roots may excrete substances such as organic acids that complex with Al or Mn. As a result, they increase the availability of some mineral nutrients more to roots such as P, and may decrease the activity of others that inhibit plant growth such as Al and Mn. Nevertheless, contradictory results exist in the literature of AMF on the function in increasing or decreasing the uptake of some elements such as Al and Mn. According to Nogueira and Cardosso (2003), the effect of AMF may vary from beneficial to adverse or indifferent depending on the type of AMF, soil type and the stage of plant development.

2.3. Arbuscular mycorrhizal fungi and manganese toxicity

Manganese toxicity is a serious constraint to many crops grown on certain acid soils in the world. Highly weathered soils with large amount of sesquioxide clay minerals often have a high amount of manganese (Hue et al., 2001). As a result, plants that grow on soil with high exchangeable Mn can accumulate a large amount of this element slowing their growth. Unlike Al toxicity symptoms, Manganese toxicity symptoms are located in the shoot, and are characterized by stunted growth, chlorosis, and necrotic lesions in the leaves (Kochian, 2004). Site of Mn toxicity symptoms differ depending on plant species. For example, the toxicity symptom (marginal necrosis and necrotic spots) appears on the youngest leaves on soybeans, whereas they are found on the oldest leaves in tomato.

Leucaena leucocephala Mn toxicity symptoms are characterized by marginal necrosis of older leaves, with the necrotic spots surrounded by whitish chlorotic rings. According Ruaysoongnern et al. (1989) a few black spots might be observed on the lower surface of leaves at high levels of Mn toxicity. According to the same source, a high Mn level may affect the nodule dry weights of inoculated plants. They evaluated the critical Mn concentration for toxicity associated with a 10% reduction in yield at 325 mg Mn kg^{-1}. *Acacia koa,* as well as *L. leucocephala* are susceptible to soil acidity and associated Mn toxicity. Manganese toxicity symptoms on *A. koa* are usually located on the old leaves and consistt of necrotic spots. However, few studies have assessed Mn toxicity of *A. koa* in acid soils.

Different plant species or even varieties within the same species have developed different levels of tolerance to Mn (Foy et al., 1988). The main management method to protect plants against Mn toxicity is liming, which can be costly. However, one alternative could be the use of AMF to alleviate this toxicity. Several studies have indicated the beneficial effect of AMF in alleviating the effect of Mn in acidic soils (Nogueira et al., 2004; Cardoso et al., 2002; Clark, 1997; Medeiros et al., 1994). However, the effect of AMF on Mn uptake by the host has been controversial depending on the studies involved. For instance, Menge et al. (1982) did not find any effect of AMF on Mn uptake by the host plant. Several authors (Medeiros et al., 1994; Bethlenfalvay and Franson, 1989) found a lower Mn concentration in both shoots and roots of mycorrhizal-soybean compared to non-mycorrhizal controls. In their study Medeiros et al. (1994) have noted severe symptoms of Mn toxicity and high concentrations of Mn in the leaves (314 $\mu g\ g^{-1}$) of non-mycorrhizal plants. They suggested that the higher level of

Mn was due to the exudation of organic acid in non-mycorrhizal plants that solubilizes MnO_2 and chelated Mn^{2+} facilitating absorption. They also observed an increase of pH in nmycorrhizal plant rhizosphere and an increase in exchangeable Mn in non-mycorrhizal soil. On the other hand, with low soil Mn, Menge et al. (1982) found a significantly higher leaf Mn concentration in AMF than non-AMF host plants, while in other soils with high Mn content the leaf concentration was lower. Also, Medeiros et al. (1994) found no significant difference between the shoot Mn content of mycorrhizal and non-mycorrhizal plants, although the root Mn content was lower in mycorrhizal plants than non-mycorrhizal. They also noted that visual symptoms of Mn toxicity were more pronounced in mycorrhizal plants than non-mycorrhizal ones.

Noguiera and Cardoso (2003) investigated the effectiveness of two AMF species to enhance soybean growth and their effect on Mn uptake. They found that AMF inoculation in sandy soil resulted in increased plant biomass and lower Mn in shoot and root. Pacovsky et al. (1986) came to a similar conclusion in their study on the uptake and distribution of micronutrient in mycorrhizal soybean. They reported a significant decrease (40-50%) in the Mn uptake in AMF plants compare to the control. They also stated that Mn concentration was reduced to a grater extent in leaves than in other plant parts. Moreover, Nogueira et al. (2004) concluded that the role of AMF in alleviation of Mn toxicity symptoms in soybean may depend on the phase of symbiosis development. In their experiment, evaluation during early growth of mycorrhizal plant showed toxicity symptoms. However, the alleviation of Mn toxicity was evident in mycorrhizal plants at a later period of plant growth and, was not attributable to the growth dilution effect. Nogueira et al. (2002) noticed a better growth for mycorrhizal plants at a high level of

soil Mn. The same authors also noted a net reduction of callose deposit (an indicator of plant susceptibility to Mn toxicity) in the leaves.

In summary, there is no complete understanding of the role of AMF on decreasing or increasing Mn uptake by the plant. In particular, there is little information on AMF effects on *L. leucocephala* and *A. koa*. The current study will focus on the effectiveness of AMF in protecting *A. koa* and *L. leucocephala* against Mn toxicity.

2.4. Arbuscular mycorrhizal fungi and aluminum toxicity

Aluminum toxicity is a major growth-limiting factor for plants in acid soils (Kochian et al., 2004). The primary site of Al accumulation and toxicity is the root meristem, indicating that Al interacts with actively dividing and expanding cells. Therefore, the early signal of Al toxicity symptom is the inhibition of root elongation resulting in undeveloped short and thick roots that are inefficient in absorbing nutrients and water. When pH falls below 5.0 and lower, there is an inhibition of lateral root development and in some case roots tips are killed because of the high Al concentration. Under acidic conditions, with Al toxicity roots become brown in color and branching is significantly reduced (www.fao.org/ag/ag1/ag11/prosoil/acid1.htm). However, several plant species or cultivars within the species have developed some level of tolerance to Al toxicity.

The adaptation of legume species to soil acidity varies. *Acacia koa* and *L. leucocephala* like most legume trees are sensitive to acid soils with Al toxicity. Besides the deleterious effect of Al toxicity on their root development, Ruaysoongnern (1989) found that nodulation of *L. leucocephala* can be affected at the concentration of 5 μM

monomeric Al in the soil solution. He found that the nodulation process in legume is often more sensitive to the Al toxicity than plant growth. However, some varieties in Hawaii such as K29, K132 and K420 may be tolerant to a certain extent to acidity (Brewbaker et al., 1985).

Few studies have addressed the effects of acid soil with high Al level on *A. koa*. As for most legume species, it is expected that Al toxicity will be a significant impediment for *A. koa* growth.

Lime application has been the most common way to increase soil pH and, consequently to reduce the amount of Al in soil solution. However, some plants species or cultivars within species have developed, during their evolution, a certain level of tolerance to Al. According to Cuenza et al. (2001), Al tolerant plants, in contrast to non-tolerant plants, have the ability to grow and survive in acid soil high in soluble Al. Often tolerance to Al toxicity has been associated with the colonization of those plants by AMF.

Several authors have found a protective effect of AMF against Al toxicity. Borie and Rubio (1999) examined the effects of AMF (*Glomus etunicatum*) and liming ($CaCO_3$) on growth and mineral acquisition of Al-tolerant and Al-sensitive barley cultivars (*Hordeum vulgare* L.); they found that colonized Al tolerant barley cultivars had increased root and shoot dry matter, and increased Ca, Mg and P concentrations in shoots. They also found an association with the decrease in Al uptake with increase in the rhizosphere pH. This change of pH makes the Al less available for the plant. They concluded that AMF, in comparison with lime, appeared to ameliorate Al phytotoxicity for Al-tolerant cultivars by decreasing Al/P, Al/Ca and Al/Mg ratios.

Medeiros et al. (1994) in their study on the effect of excess Al on mineral uptake in mycorrhizal (*Glomus intraradices* and *G. etunicatum*) sorghum [*Sorghum bicolor* (L.) Moench, cv. SC283] have also confirmed the role of AMF in protecting plants against Al toxicity. They found a significant decrease of dry mass (43%) of non-mycorrhizal sorghum compared to the control (0 μM Al). Thus, the AMF increased the tolerance of sorghum against Al toxicity. Cuenza et al. (2001) indicated that AMF are able to bind Al in their mycelia, and particularly in vesicles and/or in auxiliary cells. They concluded that AMF could influence Al tolerance in *Clusia. multiflora*, by decreasing the quantity of this element that is absorbed by the roots. The level of protection may depend on the provenance of the isolate AMF. The authors found that an AMF isolated from acid soil contributed more to the tolerance to acidity than that from neutral soil. In addition, Cumming and Ning (2003) attributed the significantly lower accumulation of Al in mycorrhizal broomsedge (*Andropogon virginicus*) plants to the change in Al speciation and availability in the rhizosphere as a consequence of AM fungal colonization. The authors pointed out that the analysis of leachate Al concentration indicated a lower concentration of monomeric Al in the rhizosphere of mycorrhizal broomsedge. However, they found a much lower activity of acid phosphatase (Apase), which is considered as a marker of Pi limitation. But this study did not specify the chemical mechanism involved in the change of form of Al in the rhizosphere. Clark et al. (1999) found that many AMF isolates enhance plant acquisition of mineral nutrients essential to plant growth and alleviate nutrient deficiencies encountered by the plant when grown on acidic soil.

Cumming and Ning (2003) have found that the growth of mycorrhizal plants in acid soil was less affected by Al-induced changes in the root or shoot Ca and Mg

concentrations, which suggests that AMF improved nutrient homeostasis. Similarly, Clark and Zeto (1996) found that the uptake of nutrients such as Ca, Mg and K were enhanced in mycorrhizal plants grown on acid soils, and hypothesized that this effect may have contributed to the alleviation of Al toxicity. Similarly, Borie and Rubio (1999) suggested that the lower molar ratio of Al/P, Al/Ca are indicators of the beneficial mycorrhizal effects on Al toxicity.

However the impact of AMF on Al uptake remains controversial. Some sources found an increase of Al in plant shoots (Daft et al., 1975), when others have detected a decrease (Koslowski and Boerner, 1989).

Medeiros et al. (1999) also suggested that increased P uptake by AMF plant is an Al tolerance mechanism. They reported that the form of P transported by AM hyphae is polyphosphate and that complexation of Al with that polyphosphate may contribute to the tolerance of Al toxicity. In addition, Clark and Zeto (1996) suggested that substances secreted by the hyphae such as growth promoting compounds like indole acetic acid, or abscisic acid could be mechanism of Al tolerance to Al toxicity.

In particular, it is not known whether acid tolerant AMF may play an effective role in alleviating Al toxicity for *A. koa* and *L. leucocephala*. Moreover, previous research has not explained fully the involvement of AMF in Al tolerance.

The present study will focus on the effectiveness of AMF in protecting *A. koa* and *L. leucocephala* against Al and Mn toxicities in Al toxic (Leilehua soil) and Mn-toxic (Wahiawa soil).

CHAPTER 3: Determination of the levels of aluminum and manganese toxic to *Acacia Koa* in Acid soils

3.1. Abstract

Soil acidity is often associated with aluminum and/or manganese toxicity. A greenhouse investigation was undertaken to determine the level of Al and Mn that is toxic for *Acacia koa* on two acid soils (Al-toxic soil - Leilehua series and Mn-toxic soil - Wahiawa series) at different levels of pH 4.3, 4.8, 5.2 and 6.1 for Al-toxic soil; and 4.6, 4.8, 5.2 and 6.2 for the Mn-toxic soil. In Al-toxic soil, there was a linear relationship between AMF root colonization, shoot dry matter and soil pH whereas, there was a quadratic relationship between shoot Al content, root dry matter and soil pH. In Mn-toxic soil, the changes in pH did not affect root colonization and root dry matter. However, low soil pH suppressed shoot dry matter. In conclusion, 0.64 $cmol_c$ Al kg^{-1} in Al-toxic soil and 92 mg Mn L^{-1} in Mn-toxic soil were estimated to be the toxic level of Al and Mn for *A. koa* based on the 20% reduction in shoot dry weight.

3.2. Introduction

Acid soil infertility is a major obstacle to crop production in many parts of the world particularly in the humid tropics (Uexkull and Muter, 1995). The infertility of these soils is mainly due to the low level of nutrients such as phosphorus (P), calcium (Ca), magnesium (Mg) and toxicities of aluminum (Al) and/or manganese (Mn). High Al content in an acid soil interferes with root system development (Kochian et al., 2004), that results in a limited water and nutrient uptake (Barcelo and Poschenrieder, 2002; Jones and Kochian, 1995).

In addition, Mn toxicity may be a serious constraint to many crops grown on certain acid soils in some parts of the world. Plants that grow in soils with high levels of exchangeable Mn can accumulate a large amount of this element that slows their growth (Hue et al., 2001, Bethlenfalvay, 1989). Unlike Al toxicity symptoms, that appear first on roots, Mn toxicity symptoms are localized in the shoot, and are characterized by stunted growth, chlorosis, and necrotic lesions on leaves (Kochian et al., 2004).

Acacia koa A. Gray is a fast growing leguminous tree that forms mutualistic associations with nitrogen fixing bacteria (Rhizobium). It is endemic to Hawaii'i and is often used in Hawaii'i to reforest abandoned agricultural lands. Its growth may be inhibited by low fertility and toxic concentrations of Al and Mn in acidic Oxisols and Ultisols (Scowcroft and Silva, 2005). A*cacia koa* has been prized for its excellent wood quality and also for its cultural and economical significance for the islands. Despite its high economic value, few studies have measured the level of Al and Mn in acid soil that can be toxic to *A. koa*. The aim of the current investigation is to determine the levels of Al and Mn that are toxic to *A. koa* in acid soils.

3.3. Materials and Methods

3.3.1. Soil preparation

An Al-toxic Ultisol (Leilehua series, Very-Fine, Ferruginous, Isothermic Ustic Kanhaplohumult) and a Mn-toxic, moderately weathered Oxisol (Wahiawa series, Very-Fine, Kaolinitic, Isohyperthermic Rhodic Haplustox) were selected for the study. The Leilehua soil was collected from the Waiawa Correctional Facility (WCF) on the island

of Oahu and the Wahiawa soil was collected from the University of Hawaii Experimental Farm, Poamoho, and Oahu, HI. The soils were collected from a depth of 0 – 15 cm and sieved to pass through a 4 mm aperture sieve. The initial pH measurements (1:2 soil: water) of were 4.8 and 5.3 for the Leilehua and Wahiawa soils, respectively. Portions of the soils (2.25 kg) were transferred into 2.5 L plastic pots and limed with dolomite or acidified to desired pH values (4, 4.5, 5.0, and 6.2). To acidify the Leilehua soil from pH 4.8 to 4 and 4.5, a 0.4% and 0.22% solution of H_2SO_4 was used to bring the soil to its maximum water holding capacity. Following soil properties were determined after adjusting soil pH: KCl-extractable Al and Mehlich 3-extractable Mn. Soil properties are presented in Table 3.1.

Table 3.1. Selected properties of Al-toxic (Leilehua) and Mn-toxic (Wahiawa) soil.

Soil	pH	Ca*	Mg*	Al**	Mn†
			$cmol_c\ kg^{-1}$		($mg\ L^{-1}$)
Al-toxic	4.3	1.87	2.23	4.10	30
	4.8	1.38	1.59	3.60	16
	5.2	3.00	3.42	0.64	8.5
	6.1	3.13	3.59	0.14	6
Mn-toxic	4.6	1.69	1.83	3.08	259
	4.8	1.57	1.82	2.96	124
	5.2	3.10	2.58	0.22	92
	6.2	6.93	7.59	0.13	37

*Exchangeable Ca and Mg (1 *M* KCl)

**KCl-extractable Al

†Mehlich 3-extractable Mn

3.3.2. Seed preparation

Seeds of *A. koa* used for the present experiment were collected at 2000 m on the island of Maui. Scarification was achieved by immersing seeds in concentrated sulfuric

acid for 30 minutes. The seeds were then washed 4 times using sterilized water. The scarified seeds were placed on a sterile moistened paper towel in petri dishes and incubated at 28 °C in the dark for 3 days. Uniformly germinated seeds were selected and planted in multi-well trays filled with calcined montmorillonite (Turface™). The seedlings were grown for 35 days before they were transplanted into the pots containing the test soils.

3.3.3. Transplanting of seedlings

Seedlings were washed free of Turface. Transplanting of seedlings into soil was achieved after wetting the soils to the field capacity. A 15 cm hole was made in the center of each pot with forceps and one seedling per pot was placed in the depression.

3.3.4. Measurements

After 8 weeks of growth, plants were harvested. Shoots were severed at soil level and their dry matter was determined after oven-drying at 70 °C until constant weight was obtained.

Roots were carefully washed with high-pressure water. The percentage of root colonization was determined on a 0.5 g portion of root sample. The root samples were cleared and stained in order to observe the diagnostic features of AMF. This process consisted of immersing root samples into a 10% KOH solution for 24 hours in order to clear roots. After rinsing 4 times with de-ionized water, roots were acidified for 5-10 min in 10% HCl in order to facilitate the retention of the staining material. After removing the acid, roots were stained by covering them with a fuchsin-lactic acid solution and

incubated for 24 hours then destained by incubating in a destaining solution at (22° C) for 24-48 hours (Habte and Osorio, 2001). The stained roots were distributed in petri plate with grid markings and evaluated under a stereoscopic microscope using the gridline intersect method to assess AMF colonization of roots (Giovanetti and Mosse, 1980).

The experiment was carried out at the University of Hawaii College of Tropical Agriculture and Human Resource (CTAHR) Mauka green house under natural light. (21° 18' 23.2" N and 157° 48' 36.14" W). Treatments were arranged on benches in a randomized complete block design with four replicates per treatment. The irrigation system was set to water plants 3 times a day for 5 min each. Plants were grown from March 15, 2007 to May 18, 2007.

Root colonization, shoot dry matter yield, root dry matter and shoot Al and Mn were statistically analyzed using the version 9.1 of the SAS software (SAS Institute, 2000). Analysis of variance (ANOVA) was performed on the data, and regression analysis was used to evaluate the effects of pH on the different variables under study.

3.4. Results

3.4.1. Al-toxic soil

3.4.1.1. AMF colonization of roots

There was a significant linear relation between soil pH and root colonization ($p = 0.0133$) (Fig 3.1). For each unit increase of pH, root colonization of *A. koa* by indigenous AMF increased by 7.05 %.

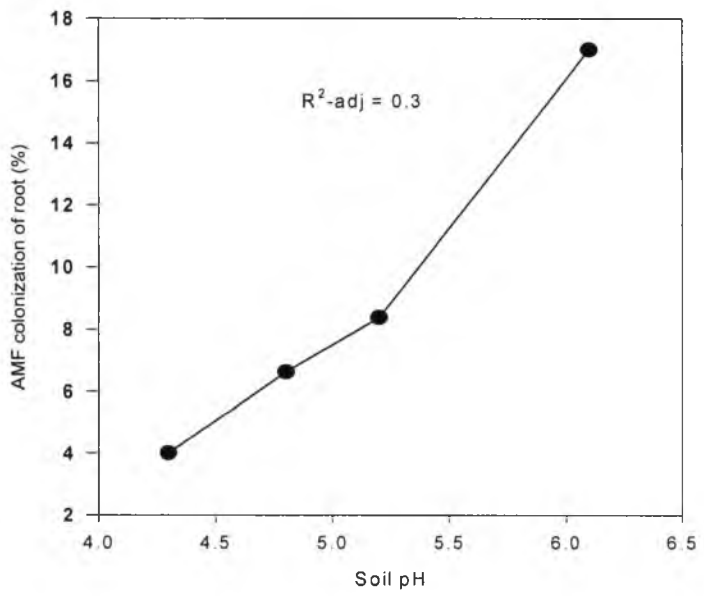

Figure 3.1. Effect of soil pH on *A. koa* root colonization in an Al-toxic soil.

3.4.1.2. Shoot dry matter

Data showed a significant linear relationship between shoot dry weight of *A. koa* and soil pH ($p = 0.0001$). Shoot dry matter of *A. koa* increased by 5.42 g for each unit increase of pH. (Fig 3.2). The growth reduction observed at pH 4.3, 4.8 and 5.2 were 68, 33 and 20%, respectively.

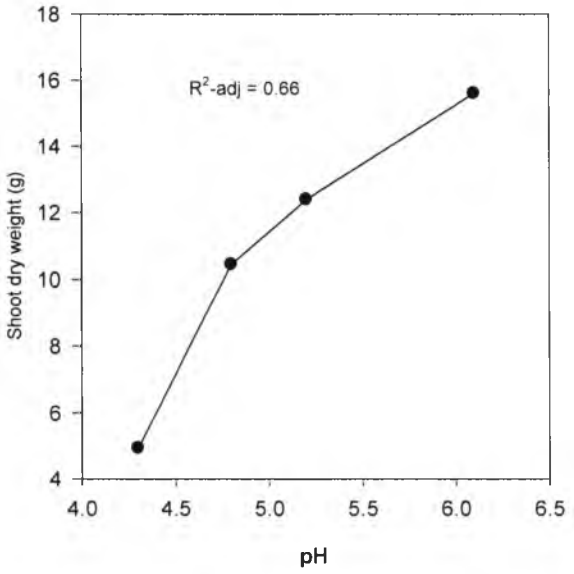

Figure 3.2. Effect of soil pH on *A. koa* shoot dry weight in Al-toxic soil.

3.4.1.3. Tissue Aluminum and manganese concentrations

There was a significant quadratic relationship between shoot Al concentration of *A. koa* and soil pH (Fig 3.3). Shoot Al content significantly decreased up to pH 5.2 (slope = 89.39 g, p = 0.0321). From pH 5.2 to 6.1, the pH did not significantly affect shoot Al content (p = 0.240).

There was also a significant linear relationship between *A. koa* shoot Mn content and soil pH (p = 0.0123). Shoot Mn content significantly decreased by 27.28 ug g^{-1} with each unit increase of soil pH (Fig 3.4).

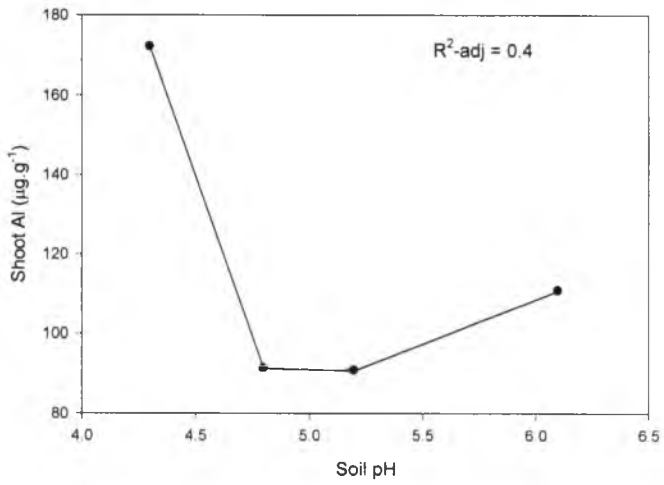

Figure 3.3. Effect of soil pH on *A. koa* shoots Al content in Al-toxic soil.

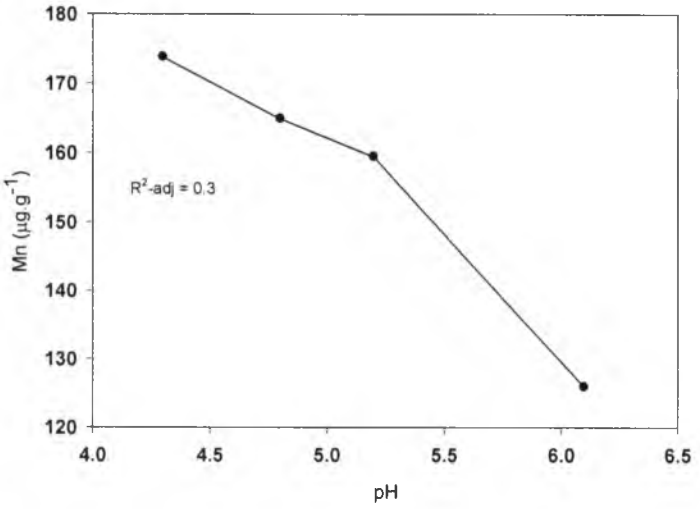

Figure 3.4. Effect soil pH on *A. koa* shoots Mn content in Al-toxic soil.

3.4.1.4. Root dry weight

There was a significant quadratic relationship between root dry matter yield and soil pH of *A. koa* in the Al-toxic. Root dry matter increased up to pH 4.8 (slope = 4. 87, p = 0.0189). Increasing pH from 4.8 to 6.1 did not significantly affect root dry matter (p = 0.1300) (Fig 3.5).

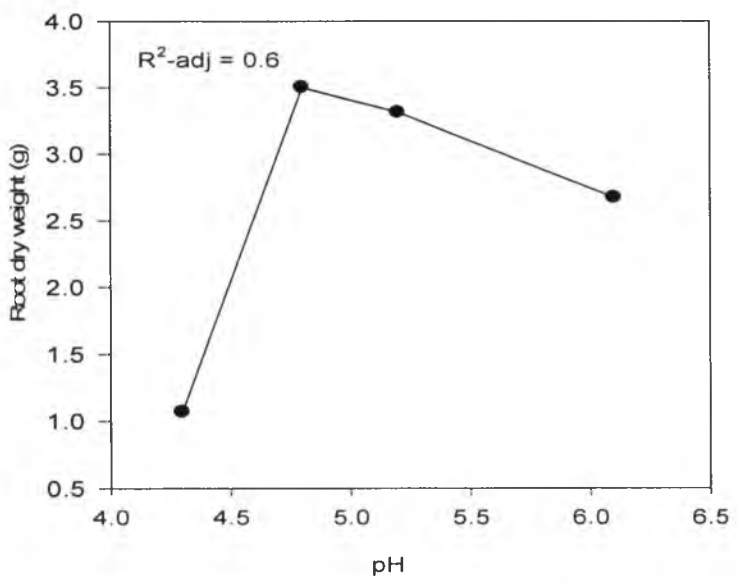

Figure 3.5. Effect of soil pH on root dry weight of *A. koa* on Al-toxic soil.

3.4.2. Mn-toxic soil

3.4.2.1. AMF colonization of roots

Colonization of roots by indigenous AMF was not significantly influenced by soil pH (p = 0.5136).

3.4.2.2. Root and shoot dry matters

In the Mn-toxic soil, changes in pH had no significant effect on root dry matter yield ($p = 0.7756$). However, shoot dry matter yield of *A. koa* was significantly suppressed at pH 4.6 compared to that observed at pH 6.2. Decreasing pH form 6.2 to 4.6 linearly decreased shoot dry matter with a slope of 3.7 g ($p = 0.0025$) (Fig 3.6). A decrease at pH levels 4.6, 4.8 and 5.2 caused a shoot growth reduction by 20%.

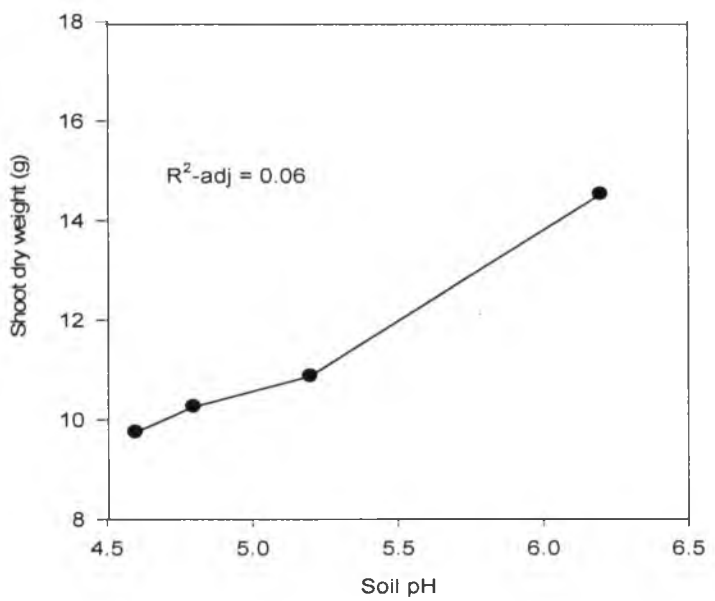

Figure 3.6. Effect of soil pH on *A. koa* shoot dry weight in Mn-toxic soil.

3.4.2.3. Shoot Al and Mn contents

Soil pH did not have any effect on Al content of roots of *A. koa* ($p = 0.4412$). However, there was a significant linear relationship between *A. koa* shoot Mn content and soil pH ($p = 0.0123$). *Acacia koa* shoots Mn content decreased by 23.52 $\mu g\ g^{-1}$ with unit of pH increase (Fig 3.7).

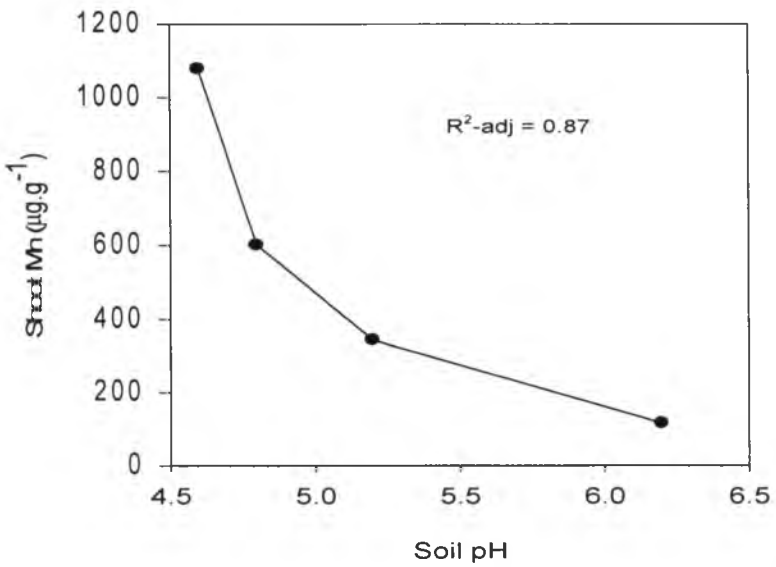

Figure 3.7. Effect of soil pH on *A. koa* shoot Mn content in Mn-toxic soil.

3.5. Discussion

3.5.1. Al-toxic soil

Acid soil associated with Al toxicity critically suppressed root colonization by AMF. One of the consequences of soil acidity and associated Al toxicity is to hamper plant growth by inhibiting the development of plant-beneficial soil microorganism interaction such as AMF (Wang et al., 1989). The low colonization observed in the current study was probably due to the combined effect of low pH, high level of Al and the low nutrient levels (Ca and Mg) (Table 3.1). Similar observations were reported by Wang et al. (1989), Ning and Cumming (2003) and Rohyadi et al. (2004). Because of liming, the availability of Al decreased and Ca and Mg contents in soil increased (Table 3.1). This condition may have stimulated higher root colonization by indigenous AMF at pH 6.1, directly impacting plant growth (Fig 3.2). As with root colonization, the linear

increase of shoot dry matter with pH increase indicated the negative effect of soil acidity on *A. koa* growth. However, the shoot growth did not coincide with root dry weight. The response of root dry weight to pH increase was in agreement with the shoot Al concentration, confirming the effect of Al toxicity on root rather than on shoot. Yost (2006, personal communication) described similar effect of Al toxicity on plants root. The decrease of shoot Al and Mn with pH increase was due to the lower availability of these elements in soil as pH increases. The decline in growth and increase in tissue Al concentration below pH 4.8 indicated that this pH was the critical point associated with Al toxicity for *A. koa*. However, at pH 4.8, the growth of *A. koa* was already reduced by 33%, which is not a tolerable loss from a management stand point. Action for correction of the detrimental effect of soil acidity needs to be taken before a substantial growth reduction.

3.5.2. Mn-toxic soil

Soil elevated level of Mn considerably reduced *A. koa* growth in Mn-toxic acid soil. As a result assessing the level of soil Mn that is toxic to the plant is very important. The lack of pH effect on AMF colonization of roots of *A. koa* suggest that either the concentration of Mn in the soil at the lowest pH tested was not high enough to suppress AM fungal activity or that the indigenous AMF were tolerant to Mn.

The suppression of shoot dry weight at low pH levels may be explained by the high concentration of Mn and the low nutrient level in the soil at these pH levels (Table 3.1). The tolerance level to Mn varies among plant species and even among cultivars. In fact, Foy et al. (1978) found that a value of 160 mg kg^{-1} of Mn in the diagnostic leaf of

soybean caused toxicity symptoms in one cultivar while, in another the critical level was 600 mg kg^{-1}. However, defining toxicity level based on plant leaf Mn concentration may lead to inaccurate conclusion when the growth factor is not taken in account (Bajita, 2003). In the current experiment, a Mehlich 3-extractable Mn content of 259 mg L^{-1} caused a growth reduction of 25% of *A. koa*. Nogueira et al. (2002) observed symptoms of Mn toxicity on soybean at soil Mn level of 20 mg kg^{-1}. However, Bajita (2003) observed high soybean yield only at soil water-extractable Mn below 2.0 mg kg^{-1} in Wahiawa soil

The effect of pH on shoot dry matter did not coincide with that of pH on root dry weight at the same pH. The lack of pH effect on root dry weight may be due to the fact that Mn toxicity symptoms appear first on plant shoot, not on roots (Marschner, 1995). In fact, tissue Mn concentration increased with soil Mn concentration (and decrease in pH) (Table 3.1) but was inversely proportional to shoot dry weight. The highest shoot Mn at pH 4.6 and 4.8 may be due to a high availability of the element in the soil at these pH levels. Horst (1988) reported that the availability of Mn is controlled by soil factors such as pH as well.

3.6. Conclusions

The results of the current investigation indicated that low soil pH seriously reduced *A. koa* growth. In Al-toxic soil, the visual toxicity symptoms were observed at pH 4.8, which corresponded to 33% growth reduction. From a management point of view, action must be taken to correct soil acidity problem before such growth reduction occurs. The levels of Al and Mn causing a 20% growth reduction were proposed as toxic

levels. Thus, 0.64 $cmol_c$ Al kg^{-1} in Al-toxic soil and 92 mg Mn L^{-1} in Mn-toxic soil were estimated to be the toxic level of Al and Mn for *A. koa.*

CHAPTER 4: Effectiveness of arbuscular mycorrhizal fungus (*Glomus aggregatum*) in increasing *Acacia koa* tolerance to Al and Mn toxicities in acid soils

4.1. Abstract

High Aluminum (Al) and/or Manganese (Mn) content in many acid soils are serious constraints to plant growth. In order to determine the effectiveness of arbuscular mycorrhizal fungus (*Glomus aggregatum*) in helping *Acacia koa* establish in low soil pH condition in soils with toxic levels of Al and Mn, a greenhouse experiment was designed and conducted. Al-toxic and Mn-toxic soils were selected for this purpose and *A. koa* was grown on them with or without inoculation of seedlings with AMF. In the Al-toxic soil, pre-inoculation of seedlings led to increases in shoot and roots dry matter, and AMF colonization of roots at low pH. In non-inoculated plants in Al-toxic soil, the increase of pH increased shoot dry matter, root dry matter, and pinnule P content. In the Mn-toxic soil, inoculation with AMF had increased pinnule P content and root dry matter mostly when soil pH was high. Inoculation with *G. aggregatum* decreased the effect of acidity by increasing the growth of *A. koa*.

4.2. Introduction

Acid soils are usually characterized by a low fertility. This low fertility is often coupled with Al and Mn toxicities. Of the deficiencies/ toxicities that plant may encounter when grown on acid soil, Al seems to be the most damaging (Foy, 1992). Aluminum is highly soluble at low pH and toxic to plants in relatively small concentrations. Aluminum inhibits both cell elongation and cell division, binds to DNA

and obstructs specific ion transport systems (Matsumoto, 2000; Kochian, 1995). As with Al, Mn is readily soluble at low soil pH and can be toxic to plants (Foy, 1992).

Liming is often used to correct the negative effect of soil acidity. However, liming may be costly and its effect is not long term depending on the management practices. Fortunately, the use of arbuscular mycorrhizal fungi (AMF) can reduce mineral toxicities and increase nutrient availability for plants grown in acid soils (Clark et al., 1999; Ning and Coming, 2003). The positive effect of AMF is partially explained by the fact that they increase the volume of soil explored by plant roots through their fungal hyphae. Mycorrhizae are believed also to increase plant resistance to mineral toxicities (Cuenza et al., 2001; Rohyadi et al., 2004; Lambais and Cardosso, 1990). However, the role of AMF in increasing plants tolerance to Al and Mn toxicities remains controversial. Some authors indicated that AMF association with plants increases the uptake of Al and Mn, whereas others have stated that the protective role of AMF is due to reducing uptake of Al and Mn (Clark and Zeto, 1996).

Many tropical soils are acid and toxic in exchangeable Al and Mn. Some tropical plant species may be tolerant to these conditions. *Acacia koa* is a fast growing leguminous tree endemic to the islands of Hawaii. *A. koa* is highly priced because of its excellent wood quality. Reforestation of some of the acid soil with *A. koa* soil is economic and environmental of interest.

The aim of this work is to evaluate if inoculation of *A. koa* with *G. aggregatum* may increase the tolerance of the plant to Al and Mn and allow its establishment on acid soils.

4.3. Materials and Methods

A greenhouse pot experiment was conducted to determine the level of protection that arbuscular mycorrhizal fungus (*G. aggregatum*) may provide to *A. koa* against Al and Mn toxicities.

4.3.1. Soil and medium preparation

The same types of soil were used in the current experiment as in Chap. 3. The initial pH for Leilehua and Wahiawa soil was 4.8 and 5.3, respectively. Portions, 2.75-kg of the sieved Leilehua and Wahiawa soil were transferred into the 3 L plastic pots and limed to the desired pH 6.2 and 6.4 respectively. The targeted pH levels were established by liming the soil based on the requirement curves constructed ahead of time. After incubation a portion of the treated soils was used to determine the 1*M* KCl extractable Al and Mehlich 3-extractable Mn in order to determine the level of these elements in the soil at fixed pH. The selected soil properties are presented in Table 4.1.

Table 4.1. Selected properties of the Al- and Mn-toxic soils.

Leilehua			Wahiawa		
pH	Al ($cmol_c\ kg^{-1}$)	Mn ($mg\ L^{-1}$)	pH	Al ($cmol_c\ kg^{-1}$)	Mn ($mg\ L^{-1}$)
4.8	3.49	35	5.3	0.67	130
6.2	0.16	13	6.2	0.19	23

The growth medium for raising seedlings was the same as the one used in Chap 3. However, in the current investigation AMF were established in seedling first before

transplanting them in the treated soil. Inoculation of the medium with AMF was achieved by incorporating 20 g of crude inoculum of arbuscular mycorrhizal *G. aggregatum*. The inoculum consisting of sand, calcined montmorillonite, spores, hyphae, and peaces of infected roots was mixed with 23 g (dry weight basis) of Canadian Premier sphagnum peat moss (PSPM) (Premier Horticulture Inc., Red Hill, Pennsylvania) (PSPM). The un-inoculated medium was prepared by mixing the same amount of inoculum carrier with the same quantity of medium.

4.3.2. Planting of seeds

Seeds were germinated as described by Habte and Manjunath (1987). Uniform-sized germinated seeds were selected and planted in plastic dibble tubes containing the mycorrhizae medium as described by Peters and Habte (2001).

The dibble tubes with seedlings were placed on racks and put under an automatic sprinkler irrigation system. The irrigation system was set to water the plants 3 times during a day for durations of 5 min each. Ten days after planting, each tube received Osmocote (19-6-12) at the rate of 12 g kg^{-1} as a source of nutrients. The time of release of the nutrient was 3 to 4 months. The fertilizer was applied on the surface of the medium. Seedlings were allowed to grow for 5 weeks before they were transplanted into pots.

The same procedure as in Chap. 3 was used for transplanting seedlings into the pots. Pots were then arranged on greenhouse benches in a completely randomized block design at the Mauka greenhouse facility (21° 18' 23.2" N and 157° 48' 36.14" W), CTAHR, and University of Hawaii at Manoa, Honolulu, Hawaii. Koa plants were allowed to grow from December 28, 2006 to March 01, 2007. Each pot received, 5 days

after planting, 200 ml of a five strength Hoagland's nutrient solution. In addition, 25 days after planting each pot received Osmocote (19-6-12) at a rate of 12 g kg^{-1} of soil. Plants were harvested after 9 weeks of growth.

4.3.3. Measurements

The effectiveness of AMF was measured as P content of *A. koa* pinnule. Pinnule P content was determined weekly as described by Habte and Osorio (2001). For this purpose, the third leaflet from the base of the youngest fully expanded leaf of *A. koa* was removed at regular intervals of time. Sampling started 30 days after planting and was repeated every two weeks thereafter. This interval of sampling depended on how fast new leaves were formed. Pinnules were dried at 70 °C for 2 h. After drying, they were transferred into 18 x 150-mm Pyrex test tubes and ashed in a furnace at 500°C for 3h (Habte and Osorio, 2001). The ash was dissolved and color developed by the molybdenum blue technique (Murphy and Riley, 1962). The concentration of P was measured in a spectrophotometer at a wavelength of 882 nm. Phosphorus concentration was expressed as μg P $pinnule^{-1}$.

At harvest shoots were cut at the soil surface and their dry matter was determined after oven drying at 70° C until constant weight was obtained. In order to determine the level of colonization by AMF, roots were carefully cleaned free of soil and the stained as described by Habte and Osorio (2001). The stained roots were spread in a petri plate marked with gridlines and were observed under a stereoscopic microscope. The percentage of roots colonization was assessed using the gridline intersects method (Giovanetti and Mosse, 1980).

4.3.4. Statistical Analysis

The data collected were statistically analyzed using the SAS software (SAS, 1991). Analysis of variance was performed to detect a significance difference between the treatments. Means within and among treatments were compared using $LSD_{0.05}$.

4.4. Results

4.4.1. Al-toxic soil

4.4.1.1. AMF colonization of roots

Despite the toxic level of Al at pH 4.8, inoculation of *A. koa* with AMF significantly stimulated root colonization ($p > 0.001$) compared to that observed in the non-inoculated plants (Fig 4.1). Changing soil pH did not influence the degree to which roots were colonized by AMF irrespective of inoculation status.

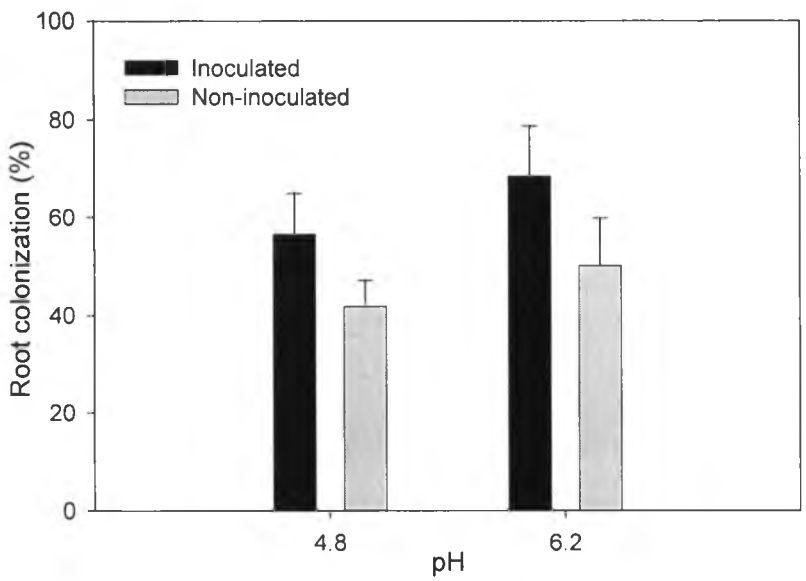

Figure 4.1. Effect of soil pH and inoculation with *G. aggregatum* on root colonization of *A. koa* in Al-toxic soil.

4.4.1.2. Pinnule P

Pinnule P content of inoculated seedlings grown on Al-toxic soil at pH 4.8 was significantly higher than that of non-inoculated plants from the 50th to the 70th days after planting (Fig 4.2a). However, low soil pH suppressed pinnule P content of *A. koa* at pH 4.8 for both inoculated and non-inoculated seedlings compared to high pH (6.2). At pH 6.2 there was no effect of inoculation on pinnule P uptake (Fig 4.2b).

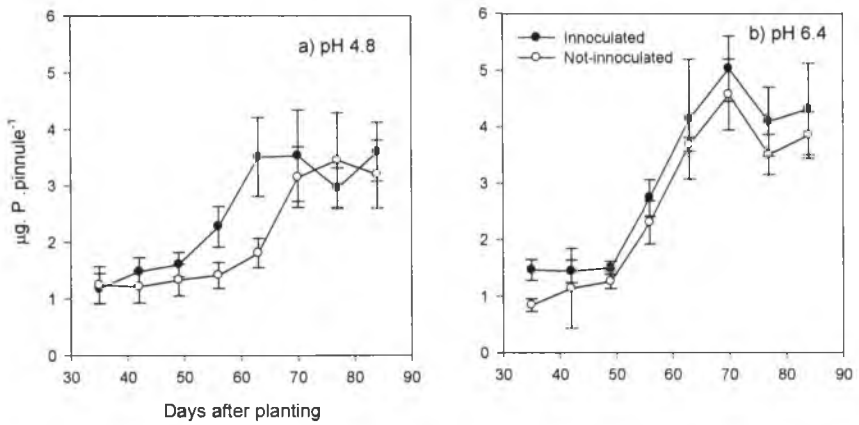

Figure 4.2. Effect of soil pH and inoculation with *G. aggregatum* on pinnule P content of *A. koa* in Al-toxic soil at pH 4.8 and 6.2.

4.4.1.3. Shoot dry matter

Acacia koa growth was significantly suppressed at pH 4.8 compared to that at pH 6.2 ($p = 0.030$), irrespective of inoculation level (Fig 4.3). However, the growth reduction of non-inoculated plants was twice as much as that observed in inoculated ones ($p = 0.020$). Inoculation with AMF increased the level of tolerance of *A. koa* to toxic levels of soil Al probably by stimulating the growth.

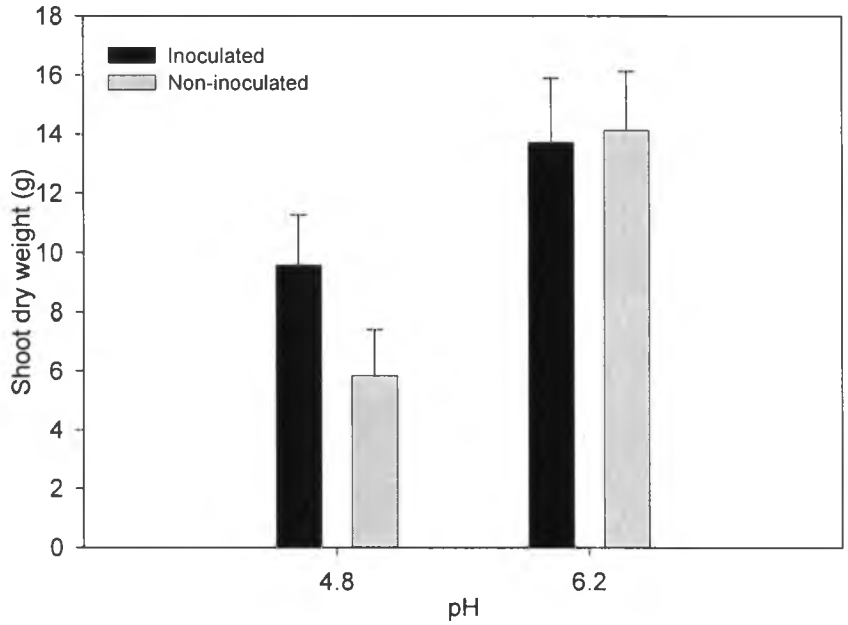

Figure 4.3. Effect of soil pH and inoculation with *G. aggregatum* on *A. koa* shoot dry matter yield in Al-toxic soil.

4.4.1.4. Root dry matter

Toxic levels of Al at pH 4.8 suppressed root development in both inoculated and non-inoculated seedlings compared to pH 6.2. However, inoculation with *G. aggregatum* did not affect root development of *A. koa* at each pH tested (Fig 4.4).

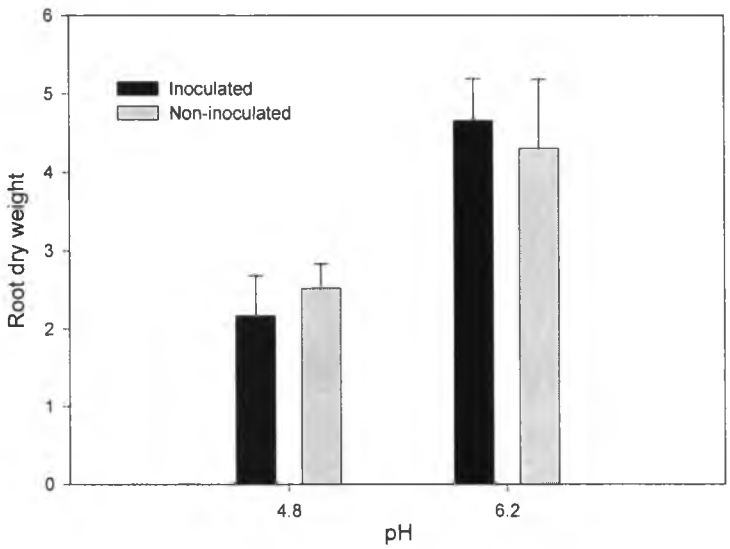

Figure 4.4. Effect of soil pH and inoculation with *G. aggregatum* on *A. koa* root dry matter in Al-toxic soil.

4.4.1.5. Shoot/root ratio

Inoculation significantly increased shoot/root ratio of *A. koa* at low pH (pH 4.8), whereas at pH 6.2, it did not affect the ratio (Fig 4.5). There was no pH effect on shoot/root ratio on non-inoculated plants. However, at pH 6.2 the ratio was lower for inoculated plants compared to that observed at pH 4.8.

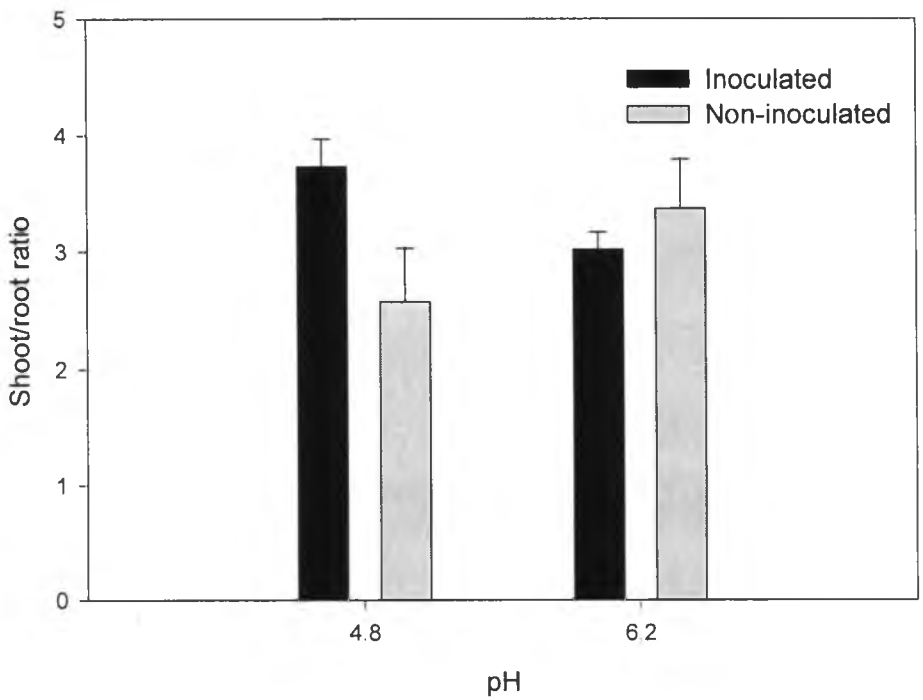

Figure 4.5. Effect of soil pH on shoot/root ratio of *A. koa* on Al-toxic soil.

4.4.2. Mn-toxic soil

4.4.2.1. Root colonization

Inoculation of *A. koa* with *G. aggregatum* at pH 5.3 significantly stimulated root colonization ($p = 0.001$). Colonization in inoculated plants was 114% higher than that of non-inoculated plants (Fig. 4.6). In addition, the effect of inoculation of *A koa* at low pH was about 3 times higher (about 65% for inoculated plants compared to 35% for non-inoculated ones) than that of liming (about 46% for non-inoculated plants at pH 6.4 compared to 35% for non-inoculated ones at pH 5.3). Increasing pH led to increase in AMF colonization in both inoculated and non-inoculated plants. There was no interaction between pH and AMF.

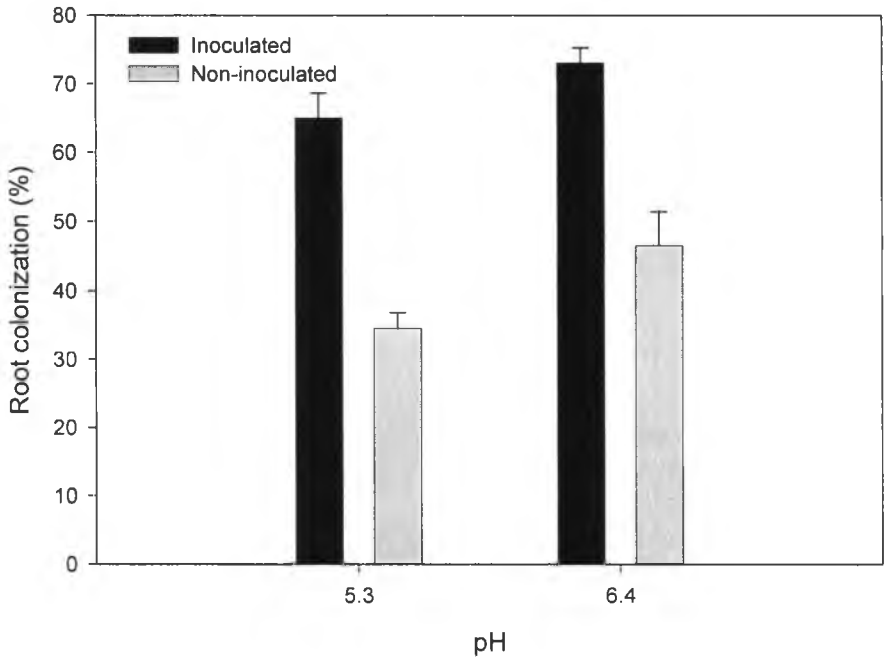

Figure 4.6. Effect of soil pH and AMF inoculation on *A. koa* root colonization in Mn-toxic soil.

4.4.2.2. Pinnule P content

Inoculation with *G. aggregatum* significantly increased pinnule P uptake of *A. koa* at pH 6.4 (Fig 4.7b) whereas at pH 5.3 there was no difference between inoculated and non-inoculated plants (Fig 4.7a).

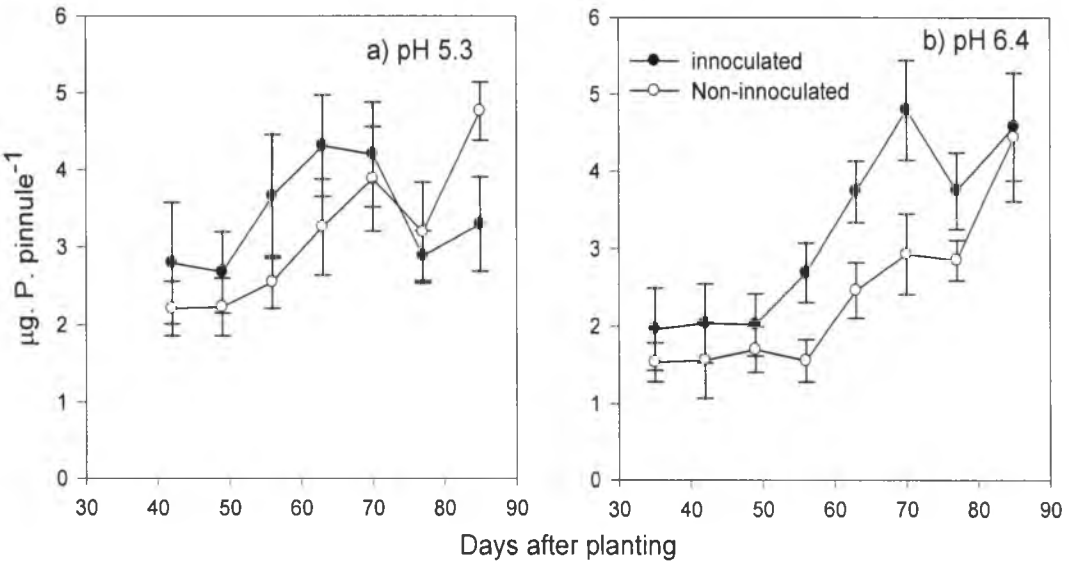

Figure 4.7. Effect of soil pH and inoculation with *G. aggregatum* on pinnule P content of *A. koa* in Mn-toxic.

4.4.2.3. Root dry matter

In non-inoculated plants, pH change did not significantly affect root dry matter (Fig 4.8). Liming the soil led to an increase of root mass in the Mn-toxic soil if plants were inoculated with *G. aggregatum*. At pH 5.3, there was no difference between inoculated and non-inoculated plants with regard to root dry weight.

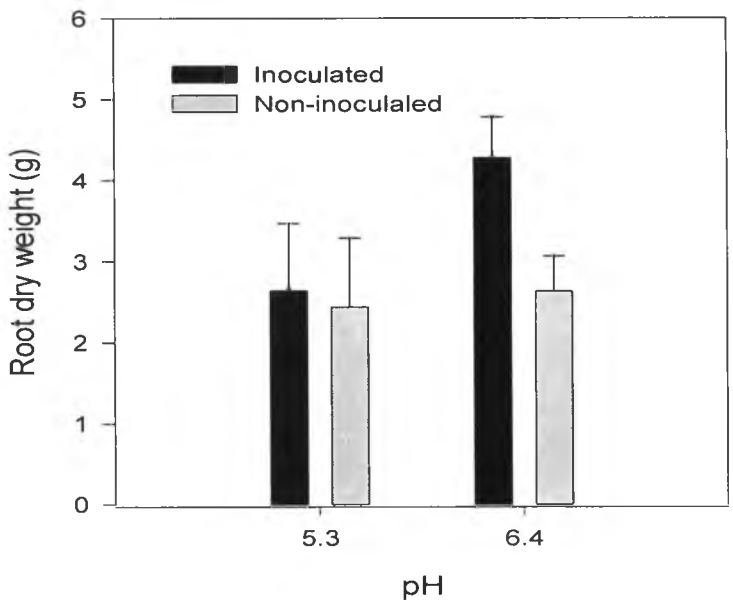

Figure 4.8. Effect of soil pH and AMF inoculation on *A. koa* root dry weight in Mn-toxic soil.

4.4.2.4. Shoot dry matter

There was no pH or inoculation effect on the growth of *A. koa* in Mn-toxic soil.

4.5. Discussion

4.5.1. Al-toxic soil

Mycorrhizal fungi played a significant role in reducing the effect of soil acidity on *A. koa* growth. In fact, the high pinnule P content in plants inoculated with *G. aggregatum* compared to non-inoculated ones at pH 4.8 was related to the higher colonization rate of inoculated seedling roots. This higher colonization rate observed in inoculated plants at low pH suggests that *G. aggregatum* might be less sensitive to low pH than indigenous AMF. The high colonization rate may have also caused the formation

of a greater quantity of external hyphae for better mining of P. The lack of difference in pinnule P content between inoculated and non-inoculated plants at pH 6.2 was probably due to a better availability of P with pH increase (Marschner, 1991; Ning and Cumming, 2003). Several authors have attributed mineral toxicity alleviation and increasing nutrient availability for plants grown in acid soils to AMF (Clark et al., 1999; Benthlenfalvay, 1992; Ning and Coming, 2003).

The physiological impacts of Al on plants are multiple, with Al interfering with cell division, retarding root growth and altering ion transport system (Matsumoto, 2000; Kochian, 1995). As it has been noted in numerous studies, suppression of root growth was also observed at high levels of Al in current experiments irrespective of AMF inoculation. This reduction of root growth was probably due to high concentration of Al pH (Table 4.1).The lack of effect of inoculation on root dry weight at pH 4.8 was not related to the suppression of root colonization. This lack of relation between root weight and colonization level was probably due to the fact that colonization with AMF may have affected root length or size without affect the weight. This may be explained by the fact that one of plant mechanism to tolerate soil nutrient deficiency condition is the decrease the shoot/root ratio (Marshner, 1995). However, our results support that *A .koa* inoculation with *G. aggregatum* increased the shoot/root ratio of inoculated plants at low pH which, indicated that the stresses related to soil low pH were, to some extent, alleviated by inoculation.

Numerous studies have associated the normal growth of plants in Al-toxic soil to the formation of symbiotic association of these plants with AMF (Clark and Zeto, 1996, Soedarjo and Habte, 1996; Cumming and Ning, 2003; Borie et al., 2002). Arbuscular

mycorrhizal fungi are known to increase uptake of nutrients such as P, Zn and Cu. In addition, some studies noted their positive role in decreasing mineral toxicities such as Al. In our experiment, the higher shoot dry matter yield of inoculated *A. koa* compared to the non-inoculated legume at low pH can be explained by the effectiveness of *G. aggregatum* in alleviating Al toxicity and improving P uptake by *A. koa.* This alleviation of Al toxicity may have been caused by the accumulation of the element in the exo-structure of the mycorrhizae (Cuenza et al., 2001).

The high shoot dry matter in inoculated plants at pH 4.8 could be also explained by high root colonization. This fact can probably be explained by the increase of P uptake at that pH due to AMF colonization (Clark and Zeto, 1996). The high colonization of root, pinnule P content and shoot dry matter indicated that inoculation with *G. aggregatum*, compared to indigenous AMF, stimulated better *A. koa* growth.

4.5.2. Mn-toxic soil

Acacia koa inoculation with AMF critically increased plant tolerance to Mn stress in acid soil. Our results revealed a higher colonization rate of inoculated plants compared to non-inoculated ones at low pH (pH 5.3). Nogueira et al. (2002) in their study of manganese toxicity in mycorrhizal and P-fertilized soybean plants reported similar results. The suppression of root colonization may have been caused by the excessive Mn which can inhibit spore germination and delays mycorrhizal formation (Koomen et al., 1990).

The increase in pinnule P in inoculated plants compared to non-inoculated ones is probably due to the positive contribution of inoculation with *G. aggregatum*. This high

pinnule P observed in inoculated plants can be explained by the higher root colonization observed at pH 6.4. However, root colonization was not consistent with the pinnule P and root dry weight at pH 5.3. Root colonization was higher in inoculated plants whereas, there was no difference between inoculated and non-inoculated plants with regard to pinnule P and root dry weight. This inconsistency between root colonization and pinnule P may due to the fact that root colonization was assessed at harvest whereas, pinnule P was monitored throughout the trial.

The lower root dry weight in inoculated plants at low pH compared to that observed at high pH may be due to the higher Mn content in the soil at pH 5.3 (130 mg L^{-1}). Nogueira et al. (2002) in their study of manganese toxicity and callose deposition noted a decrease of root dry matter with the increase of soil Mn. The fact that there was no difference between inoculated and non-inoculated plants at pH 5.3 was unexpected, since AMF are know the increase plant growth in stress condition. The decrease of Mn in soil due to the increase of pH could explain the increase of root mass at pH 6.4. This low level of Mn in soil may have lead to the increase of AMF effectiveness at pH 6.4. Toxic levels of Mn in soil can be detrimental to plant growth in acid soils. However, several authors attributed alleviation of Mn toxicity to plant association with AMF (Clark et al., 1999; Cuenza et al., 2001). The lack of difference between inoculated and non-inoculated plants in terms of shoot and root dry matter at pH 5.3 was not related to the lack of AMF colonization. The discrepancy may be due to the high variability observed in pinnule P content and shoot dry matter data. Arbuscular mycorrhizal fungi increased *A. koa* tolerance to low pH in Mn-toxic soil despite no perceptible increase of shoot dry matter.

4.6. Conclusions

4.6.1. Al-toxic soil

In this experiment, inoculation of *Acacia koa* with *G. aggregatum* ameliorated the effect of Al on root colonization, pinnule P content and biomass accumulation in acid soil. The significant difference between the biomass of inoculated plants compared to non-inoculated ones indicated that AMF conferred some degree of tolerance to soil acidity related stresses. In fact, the increase of growth due to AMF inoculation in acidic condition was equivalent to half of that attributed to liming. Therefore, in situations where high soil Al concentration is a concern, inoculation with an AMF may increase the success of survival of seedlings during reforestation.

4.6.2. Mn-toxic soil

Our study failed to show the clear positive effect of inoculation with *G. aggregatum* in increasing the tolerance of *A. koa* to Mn toxicity. Inoculation increased AMF colonization of roots, pinnule P content and root dry matter. However, because of high variability in data we failed to see the mycorrhizal effect on shoot dry matter. Further studies are needed in order to determine the effect of AMF in increasing *A. koa* to Mn toxicity.

CHAPTER 5: The effect of genotypic differences on the effectiveness of *Glomus aggregatum* in increasing *Leucaena leucocephala* tolerance to aluminum and manganese toxicities in acid soils

5.1. Abstract

High concentrations of aluminum (Al) and manganese (Mn) are two of the most important factors that limit plant productivity in acid soils. The present study was conducted in order to determine whether genotypic difference affects the extent to which inoculation of seedlings with arbuscular mycorrhizal fungus (AMF) *Glomus aggregatum* could reduce the susceptibility of *Leucaena leucocephala* to Al and Mn toxicity in acid soils. The fungus was established on roots of two cultivars (cv) of *L. leucocephala* (K8 and K636) prior to the transplanting of the seedlings in soils with toxic levels of Al or Mn at pH 4.5, 5.0 and 6.4. Arbuscular mycorrhizal colonization, measured at the end of the experiment, showed that roots of the *L. leucocephala* cultivars were colonized by AMF to a significantly greater extent at higher pH than at the lower ones. Arbuscular mycorrhizal symbiotic effectiveness measured as leaf P content indicated that symbiotic effectiveness was best expressed at the higher pH compared to the lower ones. Shoot dry matter yield of mycorrhizal plants was significantly greater than that of non-mycorrhizal ones at all pH levels. Shoot dry weight of the inoculated plants increased with the increase of pH, whereas shoot dry weight of the non-inoculated ones did not respond significantly to pH increase. Al-toxic soil significantly decreased shoot dry weight for both cultivars compared with the Mn-toxic soil. However, at a low pH, the effectiveness of AMF in protecting against acidity is expressed at a greater extent with cv K8 compared with cv K636. AMF inoculation of *L. leucocephala* at all levels of soil pH provided a certain degree of tolerance to Al and Mn toxicities in acid soils.

5.2. Introduction

Soil acidity represents one of the most limiting factors for crop production worldwide (John et al., 2005; Uexkull and Mutert, 1995). Acidic soils are characterized by a relatively high concentration of H^+ and a pH value of 5.5 or lower. However, low pH itself is often not the cause of restricted plant growth on these soils. The main constraints in acid soil are usually toxic levels of aluminum (Al), manganese (Mn) and nutrient deficiencies of Ca, Mg, P and Mo (Kochian et al., 2004). Acid soils vary significantly due to diverse factors in soil formation, especially differences in climate, parent material and vegetation type. As a result, soils that have developed under humid temperate, humid tropical and subtropical climatic conditions are usually acidic in their natural state (Habte, 1995). Many tropical soils such as Oxisols and Ultisols, that are extremely weathered fall into this category. They have high amounts of exchangeable Al or Mn due to the advanced state of soil weathering processes. Aluminum and Mn are highly soluble at low pH and may be detrimental for plants growth at a relatively low concentration. There is an important body of literature that supports the positive role of AMF in alleviating the effect of Al and Mn toxicities (Kelly et al., 2005; Clark and Zeto, 1996). However, the extent of the alleviation and the mechanisms involved remain unclear. The extent of protection may depend on the host plant and/or the variety and the environmental conditions. The objectives of the current study were (i) to determine the extent to which the toxic effects of Al and Mn on *L. leucocephala* can be alleviated by the use of AMF and (ii) to determine the extent to which Al and Mn toxicities alleviation depends on plant genotype.

5.3. Materials and Methods

5.3.1. Soils

The soils used in this experiment were the same types as in previous experiments (Chap. 3).

5.3.2. Soil preparation

The same procedure was used to prepare the soil before transplanting seedlings. For Al-toxic soil the pH levels were: 4.5 5.0 and 6.2; for the Mn-toxic soil the pH levels were 4.5, 5.5 and 6.4.

5.3.3. Plant species used and raising seedlings

The plant species used in this study consisted of two cultivars of *L. leucocephala.* The two cultivars were K636 – an acid tolerant cultivars and K8 - an acid sensitive cultivars (Blamey and Hutton, 1995).

Seedlings were first pre-colonized with AMF in a medium consisted of "Turface" (calcined montmorillonite) before transplanting them in the treated soils. Inoculation of the medium for seedling growth was achieved by mixing 20 g of crude inoculum *Glomus aggregatum* Schenck and Smith emend Koske with 70 g of Turface.

Seeds were germinated as described by (Habte and Manjunath, 1987). After germination they were grown in dibble tubes as described in Chap. 3. The levels of colonization of the two cultivars of *L. leucocephala* cv K636 and K8 were respectively 61 and 60% before transplanting. Prior to transplant seedlings in soils with different pHs,

the moisture content of the soil was raised to field capacity. A 10-inch depression was made in the center of each pot with forceps. Seedling roots were washed off Turface using high-pressure water, and they were placed in the depression.

The experiment consisted of a factorial combination of two levels of mycorrhizal inoculation (not inoculated and inoculated), two soils (Al-toxic and Mn-toxic soils) and three levels of pH (4.5, 5.0 and 6.2 for Al-toxic soil, and 4.5, 5.5 and 6.4 for Mn-toxic soil) in triplicate. Treatments were then arranged on benches in a randomized complete block design. After 10 weeks of growth, plants were harvested.

5.3.4. Measurements

The development of AMF activity was monitored by measuring pinnule P content of *L. leucocephala* leaves periodically as described by Habte and Osorio (2001). Shoots were cut off just above the soil, and dry matter was determined after oven drying at 70° C until constant weight was obtained. Roots were carefully cleaned free of soil with high-pressure water. The degree of colonization of roots by AMF was determined on a 0.5 g portion of root stained as described by Habte and Osorio (2001). Afterward, the stained roots were spread in the petri plates and observed under microscope and the colonization rate was assessed using the gridline intersect method (Giovanetti and Mosse, 1980).

5.3.5. Statistical Analysis

Arbuscular MF colonization of roots, pinnule P content and shoot dry matter yield were analyzed statistically using the SAS software (SAS institute, 1991). In order to compare the effectiveness of the AMF at different pH levels the difference between the

area under the curves of inoculated and non-inoculated plants in term of pinnule P over time was assessed by weighing the paper corresponding to that surface area (Table 5.3). The analysis of variance (ANOVA) was performed using the general linear model of SAS. The technique of LSD ($p<0.05$) was used to detect significant difference between means.

Table 5.1: Selected properties of Leilehua and Wahiawa soils before planting.

Leilehua					Wahiawa				
pH	Al*	Ca**	Mg**	Mn†	pH	Al*	Ca**	Mg**	Mn†
		$cmol_c\ kg^{-1}$		(mg/L)			$cmol_c\ kg^{-1}$		(mg/L)
4.5	1.92	1.38	1.59	12	4.5	1.04	1.57	1.82	147
5	0.79	3.13	3.56	7.4	5.5	2.22	3.13	3.58	27
6.2	0.21	7.10	8.93	7.5	6.4	2.22	6.94	7.59	24

*KCl-extractable Al

** Exchangeable Ca and Mg (1 *M* KCl)

†Mehlich 3-extractable Mn

5.4. Results

5.4.1. Al-toxic soil

5.4.1.1. AMF colonization of roots

In non-inoculated cv K636, the colonization rate by indigenous AMF was suppressed at pH 4.5 compared to that observed at pH 5.0 and 6.2 (Fig 5.1). However, inoculation with *G. aggregatum* considerably increased root colonization despite the low pH. In fact, root colonization in cv K636 was 71 and 80 % higher in inoculated plants compared to non-inoculated at pH 4.5 and 5.0, respectively. In addition, the inoculation

effect at pH 4.5 and 5.0 was 20% and 70% higher respectively than that of the combined effects of liming and indigenous AMF (Fig 5.1a).

Similarly, low pH (4.5) significantly suppressed root colonization of *L. leucocephala* cv K8. However, the colonization rate of inoculated plants was significantly higher than that of non-inoculated ones at the same pH. Unexpectedly, at pH 5.0 the effect of inoculation was less pronounced than that observed at 4.5. When comparing the effect of AMF to that of liming, results showed that inoculation increased root colonization rate from about 20% to 70% at pH 4.5, while liming from pH 4.5 to 6.2 increased it only from 20% to 42% for cv K8. Inoculation in acidic conditions clearly exceeded the effect of liming (Fig 5.1b).

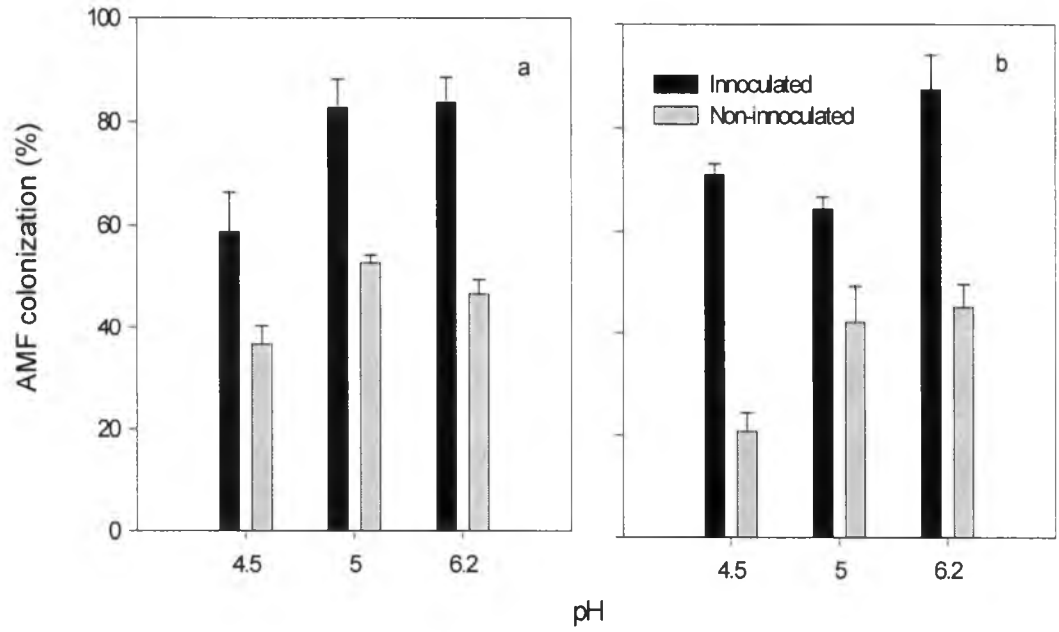

Figure 5.1. Root colonization of inoculated and non-inoculated *L. leucocephala* cv a) K636 and b) K8 at different pH levels in Al-toxic soil (Leilehua).

5.4.1.2. Pinnule P content

Pinnule P content of both cultivars of *L. leucocephala* was reduced at pH 4.5 compared to that observed at high pH levels (5.0 and 6.2) for inoculated plants (Fig 5.2). In contrast, pinnule P content significantly increased for inoculated plants compared to non-inoculated ones for both cultivars at all pH levels except for K636 at pH 4.5 (Table 5.3). For K636 at pH 4.5, there was a difference between inoculated and non-inoculated only at the beginning of the growth period (Fig 5.2a). On the other hand, *L. leucocephala* pinnule P content in non-inoculated plant did not change with soil pH. Cultivars K8 responded better to inoculation at pH 4.5 compared to cv K636 by increasing pinnule P content.

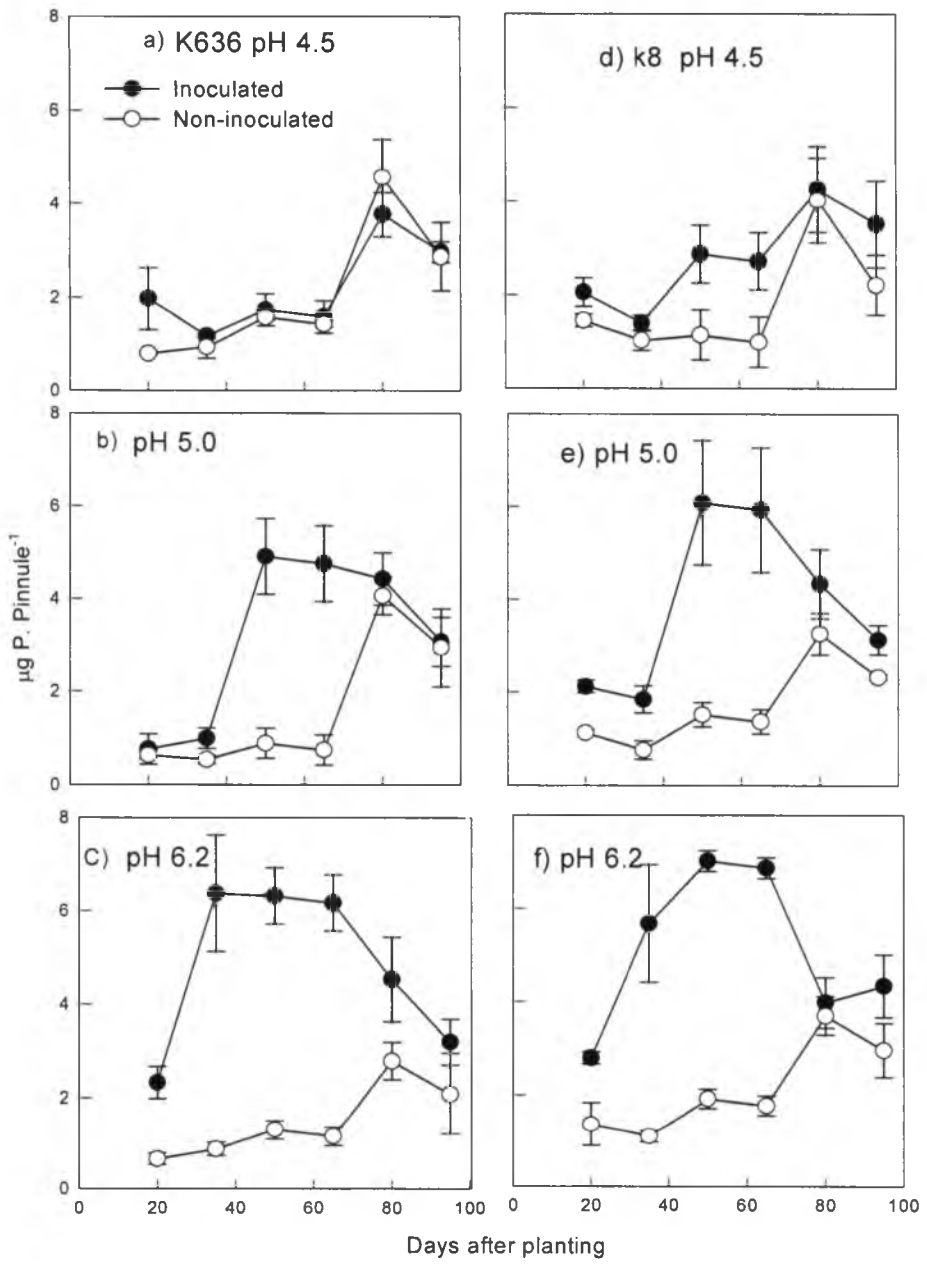

Figure 5.2. Symbiotic effectiveness of G. *aggregatum* in *L. leucocephala* cv K8 and K636 grown at different pHs in Al-toxic soil (Leilehua).

5.4.1.3. Shoot dry weight

Biomass production for both cultivars at each pH tested was improved for inoculated plants compared to non-inoculated plants (Table 5.2) (Fig 5.3). In fact, inoculation increased shoot dry weight by 43 and 175% for cv K636 and cv K8, respectively. Shoot dry weight of *L. leucocephala* cv K636 (Fig 5.3a) was significantly higher at pH 6.2 than that of *L. leucocephala* cv K8 (about 45 and 35 g, respectively for cvs K636 and K8) (Fig 5.3b). However, when grown IN low pH conditions (pH 4.5), shoot dry matter yields of the two cultivars were reduced to about 10 g compared to that of pH 6.2 (45 and 35 g, respectively for cvS K636 and K8) and 5.0 (about 20 g for both cultivars). However, shoot dry matter of the non-inoculated plants for both cultivars of *L leucocephala* was not affected by the increase in pH. There was an interaction between soil pH and AMF (Table 5.2).

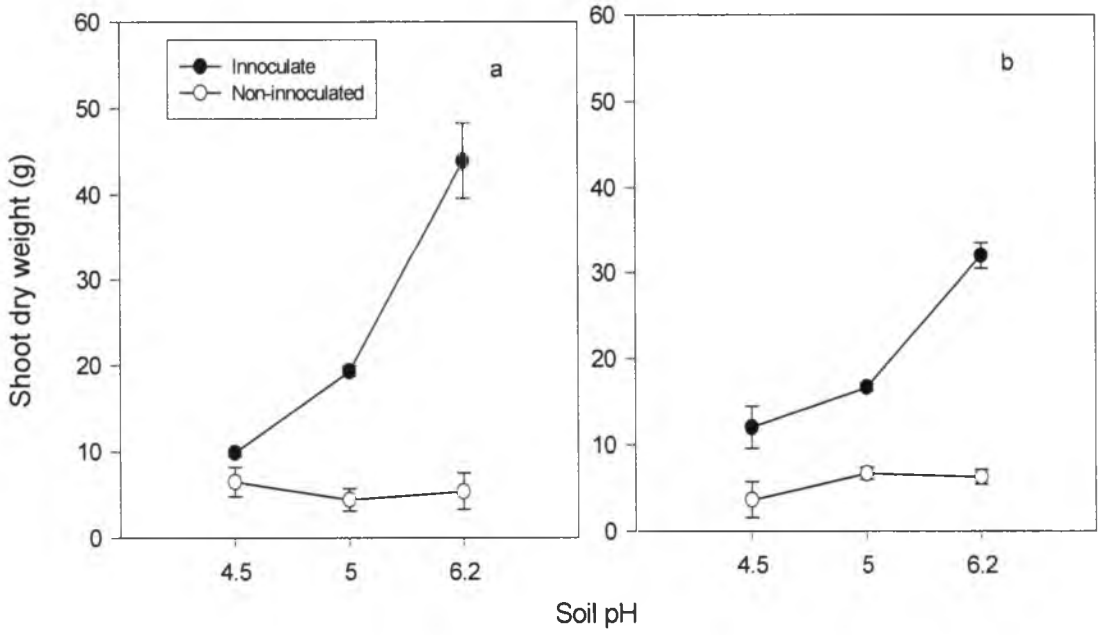

Figure 5.3. Effect of *G. aggregatum* on shoot dry matter of *L. leucocephala* cv a) K636 and b) K8 grown on Al-toxic soil at different pH.

Table 5.2. Analysis of variance for Al-toxic soil.

Variables	Factors			Interactions		
	Cultivars (CV)	pH	AMF	AMFxpH	AMFxCV	pHxCV
SDW*	NS	**	***	**	NS	NS
Colonization	**	**	***	**	**	NS

*Shoot dry weight
**significant at level 0.01
***significant level 0.001
NS: not significant at level 0.05

5.4.2. Mn-toxic soil

5.4.2.1. AMF root colonization

Root colonization in inoculated cv K636 was increased by 30, 50 and 35% at pH 4.5, 5.5 and 6.4, respectively, compared to non-inoculated plants (Fig 5.4a). However, for cv K8, root colonization increased for inoculated plants only at pH 6.4 by 60% compared to the non-inoculated ones at the same pH (Fig 5.4b). There was no cultivar effect on root colonization in inoculated plants at pH 4.5. For non-inoculated plants there was no pH effect on AMF root colonization for both cultivars (Fig 5.4). In addition, root colonization by indigenous AMF was significantly higher for K8 than for K626 at pH 4.5. There was no significant interaction between soil pH and AMF (Table 5.3).

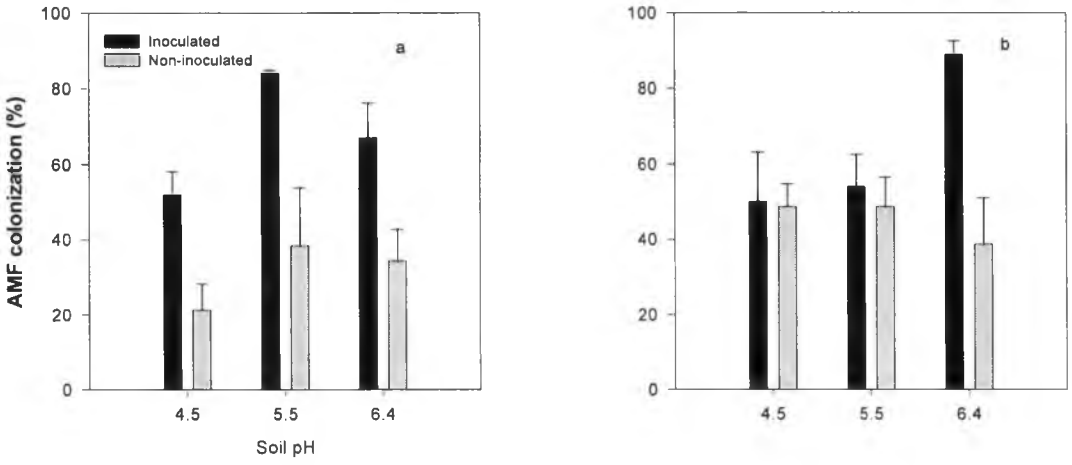

Figure 5.4. Arbuscular mycorrhizal colonization of inoculated and non-inoculated *L. leucocephala* cultivars and a) K636 and b) K8 at different pHs in Mn-toxic soil (Wahiawa).

5.4.2.2. Pinnule P content

Inoculation with *G. aggregatum* increased pinnule P content at pH 5.5 and 6.4 for cv K636 and at all pH for K8. For inoculated cv K636, pinnule P increased when pH increased from 4.5 to 5.5. Further increase from 5.5 to 6.4 did not affect cv K636 pinnule P content (Table 5.3). However, there was no pH effect on pinnule P content of inoculated cv K8. Inoculated and non-inoculated plants increased with pH for both cultivars. Pinnule P content of inoculated K8 was higher than that of inoculated K636 at pH 4.5 (Fig 5.5).

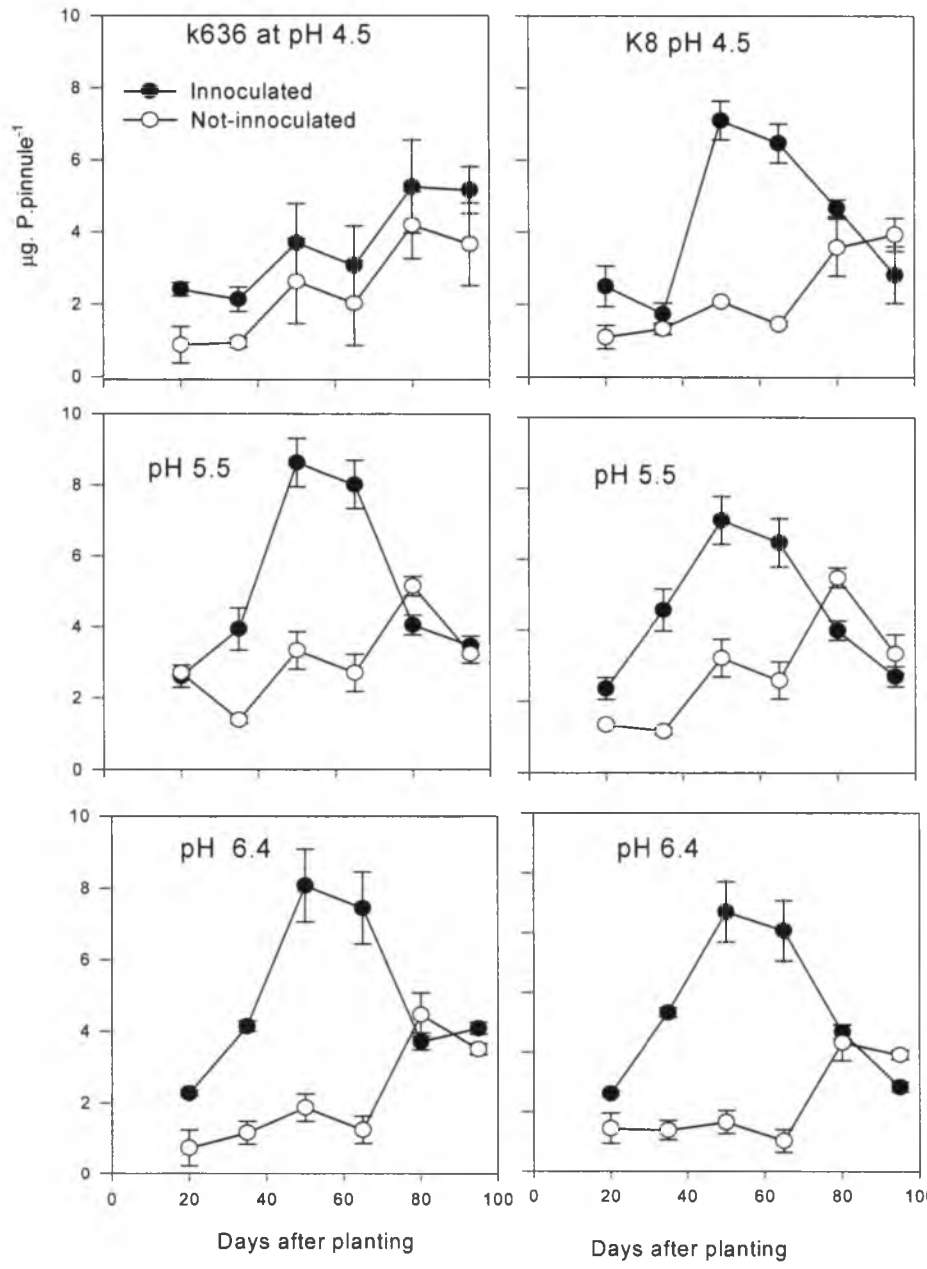

Figure 5.5. Symbiotic effectiveness of *G. aggregatum* measured as pinnule P content in *L. leucocephala* cv K8 and K636 grown at different pHs in a Mn-toxic soil (Wahiawa series).

Table 5.3. Effectiveness of *G. aggregatum* in increasing Pinnule P of *L leucocephala*. (based on Fig 5.2 and 5.5)

Leilehua			Wahiawa		
pH	Area under the curve (g)		pH	Area under the curve (g)	
	K636	K8		K636	K8
4.5	0.008d	0.021c	4.5	0.02d	0.035bc
5	0.031bc	0.047ba	5.5	0.037bc	0.036bc
6.2	0.061a	0.063a	6.4	0.059ba	0.067ba

5.4.2.3. Shoot dry weight

Inoculation with *G. aggregatum* significantly stimulated shoot dry matter at all pH levels in *L. leucocephala* cv K8 (by 140, 218 and 300%, respectively at pH 4.5, 5.5 and 6.4), whereas shoot dry matter yield for inoculated plants was stimulated only at pH 6.4 in *L. leucocephala* cv K636 (by 66%) (Fig 5.6). Shoot dry weight of inoculated plants was significantly suppressed at pH 4.5 in *L. leucocephala* cv K8 and at pH 4.5 and 5.5 in *L. leucocephala* cv K636. Increase in pH from 5.5 to 6.4 did not significantly affect shoot dry matter of inoculated cv K8. There was no interaction between soil pH and AMF in terms of their effect on shoot dry weight (Table 5.3).

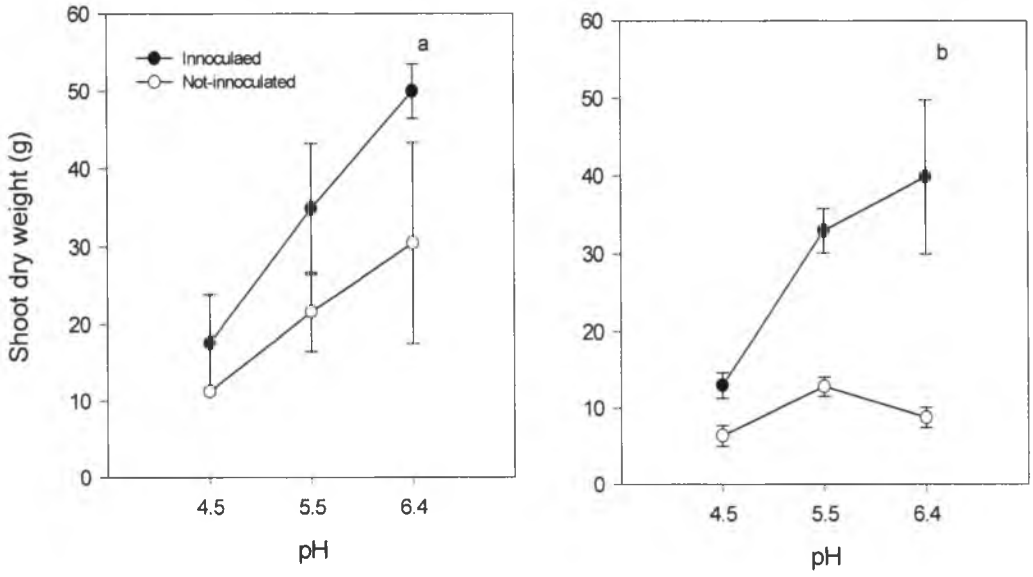

Figure 5.6. Effect of *G. aggregatum* inoculation on shoot dry matter yield of *L. leucocephala* cv a) K636 and b) K8 grown in a Mn-toxic soil at selected pHs.

Table 5.4. Analysis of variance for Mn-toxic soil.

Variables	Factors			Interactions		
	Cultivars (CV)	pH	AMF	AMFxpH	AMFxCV	pHxCV
SDW*	**	***	***	NS	NS	NS
Colonization	NS	NS	***	NS	NS	NS

* Shoot dry weight
** significant at level 0.01
*** significant at level 0.001
NS: not significant at level 0.05

5.5. Discussion

5.5.1. Al-toxic soil

Mycorrhizal symbiosis is a key factor in facilitating plants to establish in acid soil. Our finding supports this statement and showed that inoculation with *G. aggregatum* improved root colonization and shoot dry weight where root colonization by indigenous mycorrhizae was inhibited. The high root colonization rate in inoculated plants compared to non-inoculated ones can be explained by the capacity of the *G. aggregatum* to colonize *L. leucocephala* roots to a greater extent than indigenous AMF despite the toxic level of Al and detrimental conditions at pH 4.5. Habte and Soedarjo (1995) made similar observation, where they found a high colonization rate in inoculated plants at low pH compared to non-inoculated ones.

The capacity of AMF plants to promote growth in acid soils has been associated with increased acquisition of nutrient essential to plant growth especially P (Bolan, 1991; Marschner and Dell, 1994; Marschner, 1991; Cuenca et al., 2001). Numerous studies reported an increased acquisition of P by AMF plants in acid soils (Habte and Soedarjo, 1995, Clark et al., 1999; Clark and Zeto, 1996). The AMF used in our study (*G. aggregatum*) enhanced pinnule P content relative to non-inoculated plants except in cv K636 at pH 4.5. The significantly higher pinnule P of both cultivars of *L. leucocephala* when inoculated with *G. aggregatum* was related to the high level of root colonization. The high colonization rate at pH 4.5 in inoculated plants coincided with a higher shoot dry matter for both cultivars and a higher pinnule P in cv K8. However, higher colonization in inoculated cv K636 at pH 4.5 compared to non-inoculated ones did not cause pinnule P increase beyond day 35. This discrepancy may be due to the fact that

colonization of roots was measured at harvest whereas, pinnule P was monitored throughout the duration of the experiment.

The lack of pH effect on pinnule P in non-inoculated plants could not be explained by the absence of pH effect on root colonization. In fact, root colonization was suppressed at pH 4.5 in both cultivars, whereas there was no difference between pinnule P at different pH levels. The highest pinnule P observed from days 50 to 64 at pH 5.0 and from days 35 to 64 at pH 6.2 for both cultivars compared the pinnule P content at pH 4.5 may be due to better availability of nutrient and a favorable effect of high soil calcium on AMF effectiveness as suggested by Aziz and Habte (1991). However, the pinnule P content of inoculated plants were similar to or lower than that of non-inoculated plants by the end of the growing period.

The genus *Leucaena* has evolved predominantly on calcareous soils of neutral to alkaline reaction (Hughes, 1993). It is not surprising that *L. leucocepala* is often reported to be sensitive to acid soil and associated deficiency factors such low Ca and high Al and Mn content (Blamey and Hutton, 1995). The modest growth observed in both non-inoculated and inoculated plants in our experiment at low pH and the lack of response of non inoculated plants to liming indicated the sensitivity of *L. leucocephala* to soil high Al and low P, Ca and Mg and its high dependency to AMF. When comparing inoculated and non-inoculated plants at a given pH, the increase of growth in inoculated plants could be explained by a high colonization of root, high pinnule P content even at the lowest soil pH. This result is in agreement with those of Clark and Baligar (2005), where they found an increase of root colonization and P in mycorrhizal switchgrass in acid soil.

An interesting finding in our experiment was the fact at low pH, *G. aggregatum* was able to stimulate *L. Leucocephala* growth to a greater extent compared to indigenous AMF. The detrimental effect of low pH on the growth *L leucocephala* was to an extent alleviated by inoculation with *G aggregatum*. However data did not indicate a greater acid tolerance of cv K636 over K8.

5.5.2. Mn-toxic soil

Inoculation with AMF increased the level of tolerance of *L. leucocephala* to Mn toxicity in Mn-toxic soil. Manganese toxicity alleviation was one of the effects attributed to AMF in literature. This effect has been described by Marschner (1995), Nogueira et al. (2002), and Nogueira et al. (2004) and was also shown in this current study. In fact, the higher root colonization at pH 4.5 in inoculated cv K636 compared to non-inoculated ones is attributed to the capacity of *G. aggregatum* to establish a symbiotic association with that cultivar regardless of the low soil pH. Our findings are in agreement with those of a number of reports, in which root colonization of legumes was lower at low pH compared to high pH and higher in inoculated plants compared to non-inoculated ones. (Nogueira et al., 2004; Gabor et al., 1989). Inoculation attenuated the effect of low pH on root colonization by *G. aggregatum* for cv K636.

The higher pinnule P observed in inoculated cv K8 compared to inoculated cv K636 at pH 4.5, even when root colonization was higher for inoculated cv K636 than for inoculated cv K8 suggests that the symbiotic association of *G. aggregatum* with cv K8 is more effective than that with cv K636 at low pH. This better symbiotic association of *G. aggregatum* with cv K8 is supported by the increase of shoot dry matter of inoculated cv

K8 at pH 4.5 compared to non inoculated plants. The lower pinnule P content at pH 4.5 compared to that observed at pH 5.5 and 6.4 in inoculated cv K636 can perhaps be explained by the high Mn level in the soil and other unfavorable conditions related to acid soil. Nogueira et al. (2003) found that the increase of soil Mn significantly decreased the total external mycelium length in soybeans. Less development of external mycelia may have caused less mining of soil (Nogueira et al., 2003) and thus decreasing uptake of nutrients that are diffusion limited, particularly P.

5.6. Conclusions

5.6.1. Al-toxic soil

Our results clearly illustrated that inoculation with AMF alleviated the effect of low pH on *L. leucocephala* by increasing plant growth by 43 and 175% for cv K636 and cv K8, respectively. The large difference in shoot dry weight and pinnule P content between inoculated and non-inoculated plants in this study confirms the well known fact that *L. leucocephala* is highly mycorrhizal dependent species. However, the experiment failed to show the cultivars influence in increasing or decreasing effectiveness of AMF symbiotic association against soil related acidity problems. In order to maximize the benefit from AMF in acid soil with Al toxicity it is necessary to increase the soil pH.

5.6.2. Mn-toxic soil

Our study showed that inoculation with *G. aggregatum* in acid soil containing large quantities of manganese alleviated the negative effects related to soil acidity of *L. leucocephala* by increasing pinnule P and shoot dry matter (140% for cv K8). The ability

of the symbiotic association of *G. aggregatum* with *L. leucocephala* to decrease the negative effect of low pH in Mn-toxic soil on plant growth depends on the genotypic differences of plants. Increasing the pH of an acid Mn-toxic soil increased the effectiveness of *G aggregatum* in improving *L. leucocephala* growth.

CHAPTER 6: Effectiveness of arbuscular mycorrhizal fungi isolated from Leilehua and Wahiawa soils in alleviating Al and Mn toxicities for *Acacia Koa*

6.1. Abstract

High Al and Mn concentrations in acidic soils are major factors restricting plant growth. However, the plant's ability to resist Al and Mn toxicities can be enhanced by different arbuscular mycorrhizal fungal (AMF) species. A greenhouse investigation was undertaken in order to determine the effectiveness of two AMF isolates in decreasing Al and Mn toxicities for *Acacia koa* growth in Al-toxic and Mn-toxic soils. Due to a low colonization rate of seedlings at the beginning of the experiment there was no pH effect on roots colonization and shoot dry weight in Al-toxic soil. The decrease in soil pH decreased shoot Mn content and increased shoot dry matter but did not affect root colonization in Mn-toxic soil. Also the increase of pH significantly decreased shoot Mn content. In conclusion, it appeared that no significant effect due to the isolates was found.

6.2. Introduction

Acidic soils are a major problem limiting crop production worldwide particularly in the tropics (Kamprath, 1984; Uexkull and Mutert, 1995). Plants grown on acidic soils often encounter severe nutrient deficiencies (Foy, 1992) and toxic levels of Al and Mn (Kochian et al., 2004). Aluminum toxicity in acid soils is considered to be the most harmful to plant growth, because it interferes with root development and interrupts nutrient uptake (Foy, 1992). Manganese is an essential nutrient that can be toxic to plants when occurring in excess (Marschner, 1995). While liming is the traditional way to correct soil acidity, AMF are believed to increase the tolerance of their host against the

toxic levels of Mn (Nogueira et al., 2003) and Al (Clarck et al., 1999) or nutrient deficiencies. Several authors provided evidence that AMF increased mineral availability and reduced mineral toxicity of host plant grown in acidic soils (Marschner, 1991; Bethlenfalvay, 1992). Alleviation by AMF of toxicity symptoms of Al was reported by several authors (Clark et al., 1999; Cumming and Ning, 2003) and of Mn by Nogueira et al. (2002, 2003). Differences among AMF isolates in their effectiveness in alleviating the negative effect of acidic soil has been reported by Clark et al. (1999) and Kelly et al. (2005). The objective of the current study is to evaluate the effectiveness of two isolates in increasing the tolerance of *Acacia koa* to Al and Mn toxicities in acidic soils.

6.3. Material and Methods

6.3.1. Soil and medium preparation

The soils and the procedures used in the investigation were similar to the ones used in Chap. 3. The pH levels considered in the current experiment were 4.2 and 6.8 for Al-toxic soil and 4.5 and 6.6. The same medium as in previous chapters was used to raise seedling.

Inoculation of the medium with AMF was achieved by mixing 20 g of crude inoculum of AMF isolates from the Leilehua or Wahiawa soil. The inoculum consisting of sand, calcined montmorillonite, spores, hyphae, and pieces of infected roots. The crude inoculum was mixed with 23 g (dry weight basis) of (Premier sphagnum peat moss) PSPM. The non-inoculated medium was prepared by mixing the same amount of inoculum carrier with the same quantity of medium.

6.3.2. Planting of seeds

The procedures for raising seedlings and planting were similar to the ones described in Chap. 3. Seedlings inoculated with each isolates were planted and compared to the control on each soil.

6.3.3. Measurements

Arbuscular MF colonization of root, pinnule P content, root and shoot dry weights, and shoot Al and Mn contents were monitored as in Chap.3. The collected data were statistically analyzed using SAS (SAS, 1991). The analysis of variance was performed and $LDS_{0.05}$ was used to detect the significant difference between treatments.

6.4. Results

6.4.1. Al-toxic soil

6.4.1.1. AMF colonization of roots

There was no significant effect of pH on root colonization of inoculated and non-inoculated plants (Table 6.1). Inoculation of *A. koa* with the two AMF isolates (Leilehua and Wahiawa) did not significantly affect root colonization at either of the pH level tested.

6.4.1.2. Pinnule P

The increase in soil pH from 4.2 to 6.8 did not affect pinnule P content in either inoculated or non-inoculated plants for Leilehua and Wahiawa isolates (Fig 6.1). The indigenous AMF stimulated pinnule P uptake to a greater extent than the two isolates between days 50 and 65 after planting at pH 4.3. However, at pH 6.8, Wahiawa and

Leilehua isolates better stimulated P uptake than the indigenous AMF during that period. However, there was no difference in the pinnule P content between the two isolates.

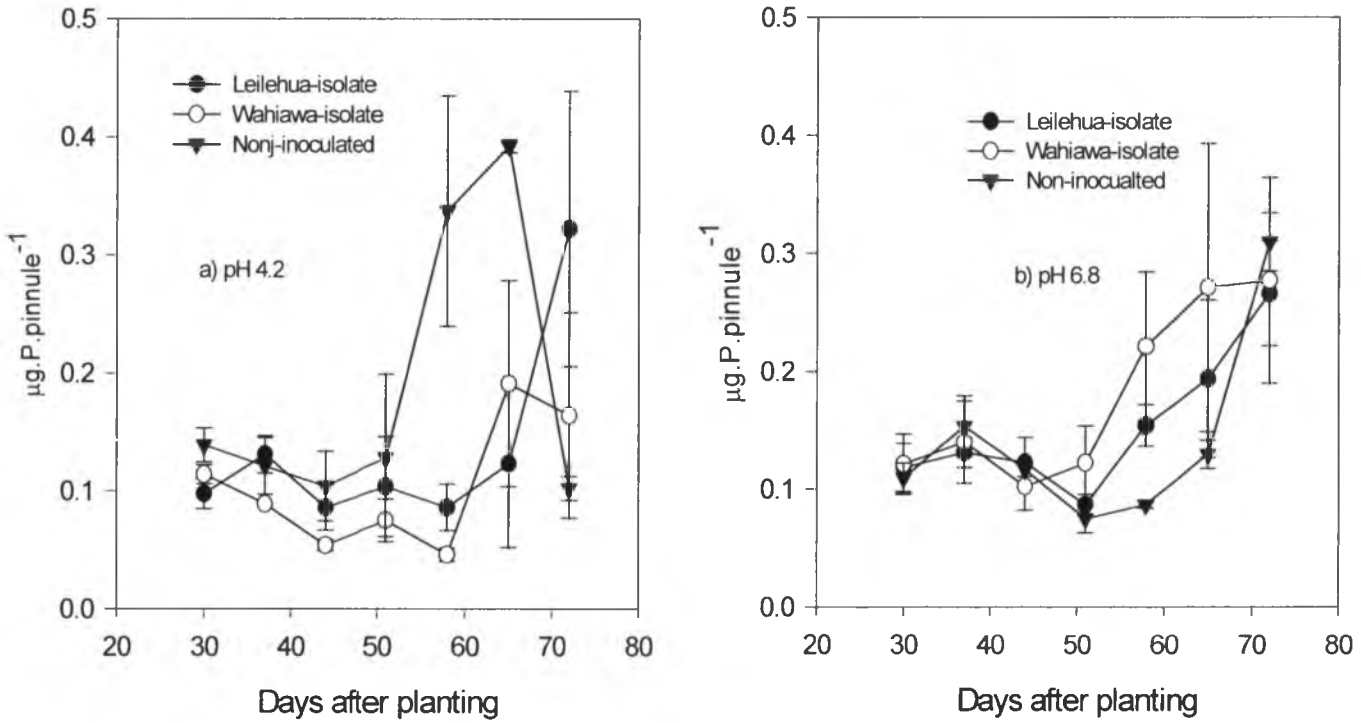

Figure 6.1. Effect of inoculation on pinnule P content of *A. koa* with Leilehua and Wahiawa isolates in Al-toxic soil at a) pH 4.2 and b) pH 6.8.

6.4.1.3. Shoot and root dry weight

In non-inoculated plants, the increase in pH did not significantly enhance *A. koa* shoot and root growth. Similarly, inoculation with either of the two isolates did not significantly affect shoot and root dry weight at each pH tested (Table 6.1).

Table 6.1. Analysis of variance of Al-toxic soil.

Trait	pH	AMF	AMF x pH
SDW†	NS	NS	NS
Colonization	NS	NS	NS
RDW±	NS	NS	NS
Shoot Al	NS	**	NS
Shoot Mn	NS	NS	NS

†Shoot dry weight
±Root dry weight
**significant level 0.01
NS: not significant

6.4.1.4. Shoot Al and Mn contents

In either inoculated or non-inoculated soils there was no pH effect. At both soil pHs, the Al content of non-inoculated plants was significantly lower than that of the two isolates which were similar. However, increasing pH decreased shoot Mn content for all inoculated and non-inoculated plants. The shoot Mn content of plants inoculated with Wahiawa isolates was statistically higher than that of non-inoculated plants and those inoculated with Leilehua isolates. Whereas there was no difference between non-inoculated plants and those inoculated with Leilehua isolates (Fig 6.2).

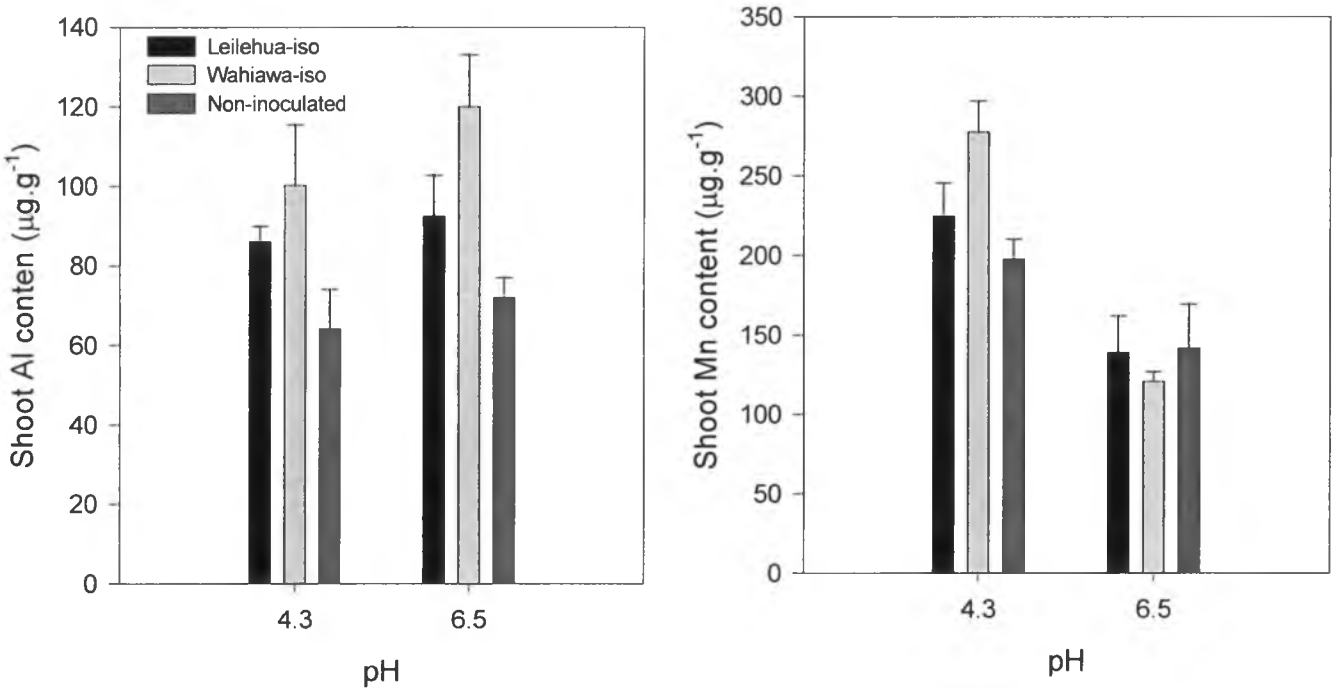

Figure 6.2. Effect of soil pH and AMF inoculation on *A. koa* shoot Al and Mn contents in Al-toxic soil.

6.4.2. Mn-toxic soil

6.4.2.1. AMF colonization of root

Root colonization of *A. koa* was not significantly affected by inoculation with Leilehua or Wahiawa AMF isolates or by soil pH (Table 6.3).

6.4.2.2. Shoot and root dry weight

Inoculation of *A. koa* with Leilehua or Wahiawa isolates did not significantly affect shoot and root growth at each pH level. The decrease of soil pH significantly suppressed *A. koa* shoot growth while it did not affect root dry matter in Mn-toxic soil. (Table 6.2)

Table 6.2. Effect of pH and AMF inoculation on root colonization, shoot and root dry matters in Mn-toxic soil.

pH	AMF status	Root colonization (%)	Dry matter	
			Shoot dry weight (g)	Root dry weight (g)
4.5	Leilehua Iso	63a	9.11b	2.64a
	Wahiawa Iso	51.75a	7.61b	1.30a
	Not-Inoculated	57.50a	10.32b	2.80a
6.6	Leilehua Iso	73.75a	13.07a	2.48a
	Wahiawa Iso	62.75a	15.11a	2.69a
	Not-Inoculated	53.75a	15.61a	2.78a

Numbers with the same letter are not statistically different.

6.4.2.3. Pinnule P

Soil pH had a significant effect on pinnule P of *A. koa* in Mn-toxic soil. At pH 4.5 the Leilehua isolate stimulated P uptake to a greater extent than the Wahiawa isolate and

indigenous AMF between 45 and 65 days after transplanting. At pH 6.6 Wahiawa isolate, stimulated pinnule P uptake to a greater extent than its counterparts starting at days 55 to 65 (Fig 6.3).

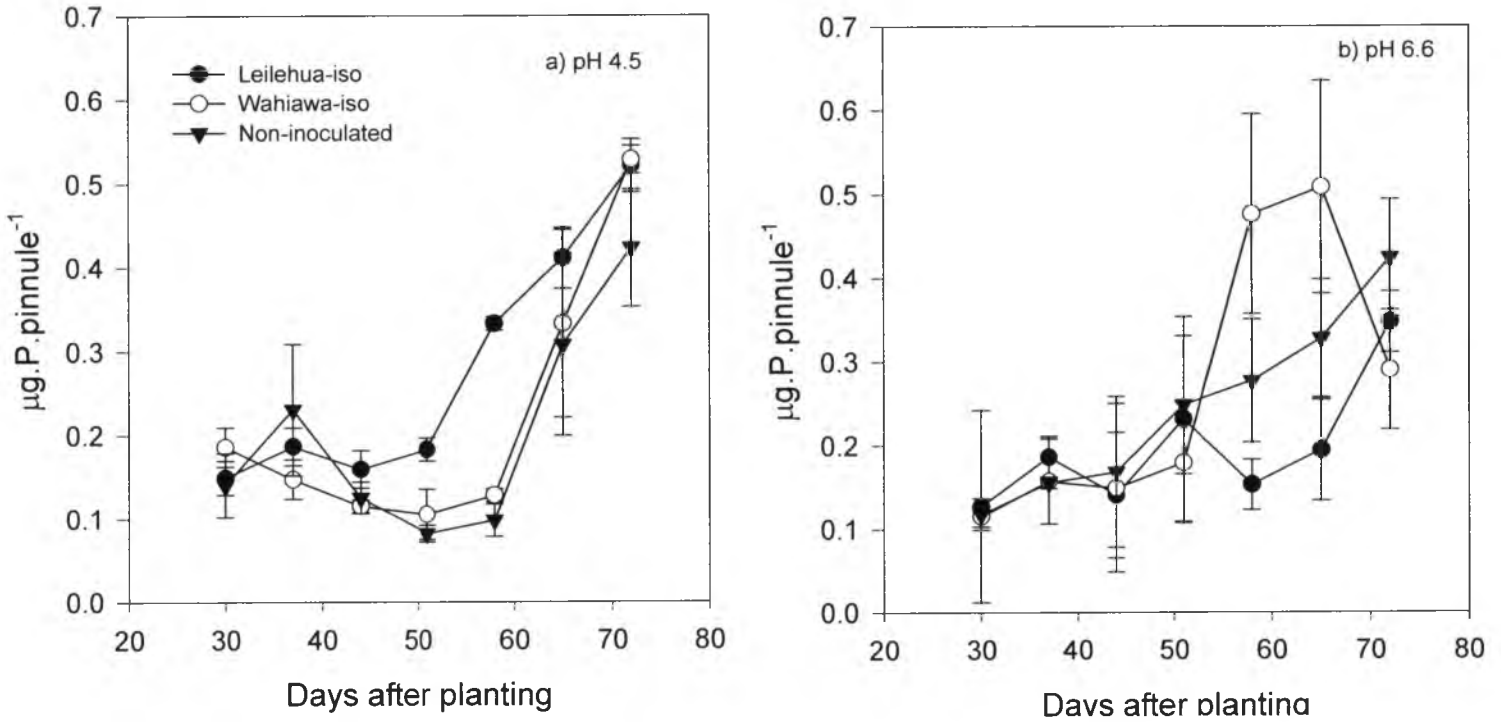

Figure 6.3. Effect of inoculation on pinnule P content of *A. koa* with Leilehua and Wahiawa isolates at pH 4.5 (a) and pH 6.6 (b) in Mn-toxic soil.

6.4.2.4. Shoot Al and Mn content

Soil pH did not significantly influence Al content (Table 6.3). However, shoot Mn content decreased significantly with increase in pH (Fig. 6.4). Shoot Al content was lower at pH 6.6 for non-inoculated plants compared to that of plants inoculated with Leilehua or Wahiawa isolates. There was no difference between the two isolates and the indigenous AMF at pH 4.5. There was also no difference in shoot Mn content among non-inoculated and inoculated plants at either pH.

Table 6.3. Analysis of variance for Mn-toxic soil.

Trait	pH	AMF	AMF x pH
SDW†	*	NS	NS
Colonization	NS	NS	NS
RDW±	NS	NS	NS
Shoot Al	NS	**	NS
Shoot Mn	***	NS	NS

†Shoot dry weight
±Root dry weight
**significant level 0.01
*** significant level 0.0001
NS: not significant

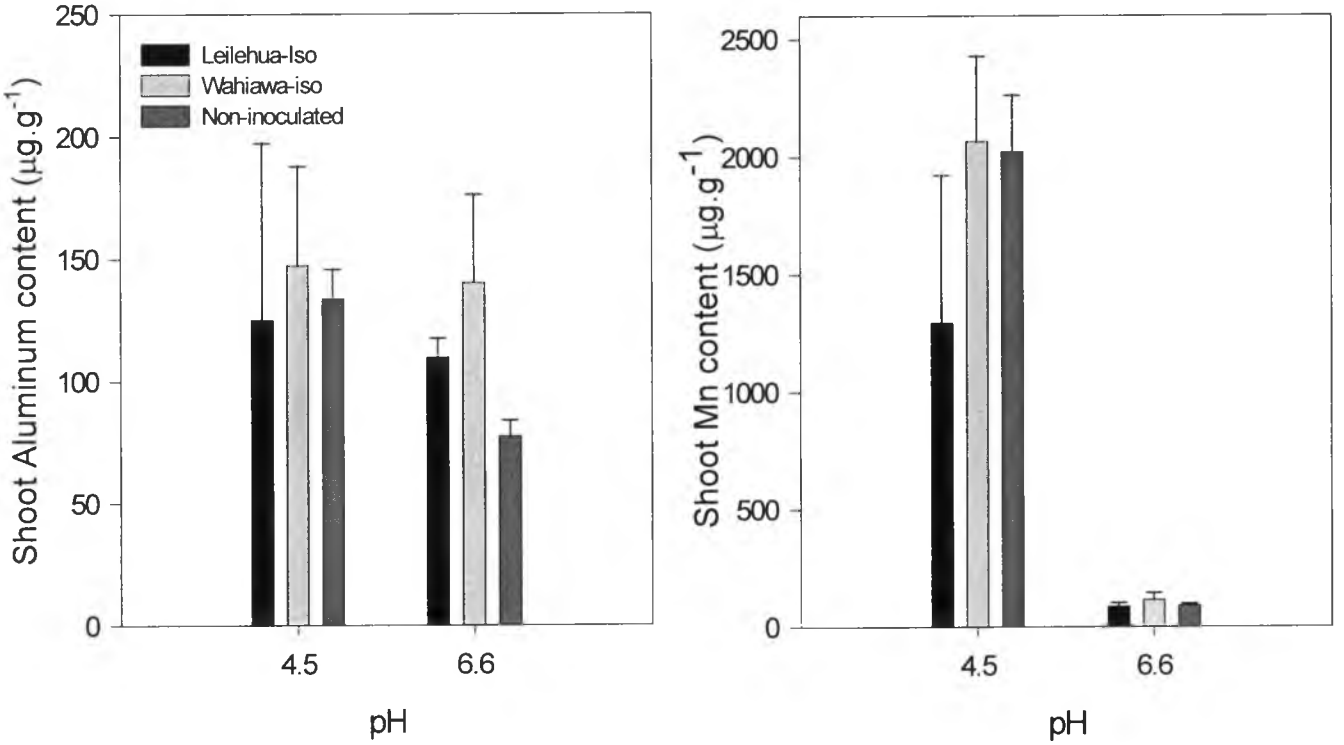

Figure 6.4. Effect of inoculation on shoot Al and Mn contents of *A. koa* with the Leilehua and Wahiawa isolates at different pH in Mn-toxic soil.

6.5. Discussion

6.5.1. Al-toxic soil

Arbuscular mycorrhizal fungi improve plant response to stresses related to soil acidity such as Al and Mn toxicities. A number of studies have documented the functional difference between AMF species, sometimes even in the same isolates (Kelly et al., 2005; Nogueira et al., 2004; Medeiros et al., 1994). The lack of difference in root colonization after harvest between inoculated and non-inoculated plants may be due to a very low initial colonization rate of *A. koa* seedlings (6 to 8%) before transplanting. However at harvest the colonization level was high. This may be explained by the increase of the activity of indigenous AMF.

The lack of effect of inoculation in stimulating pinnule P content of *A. koa* may be explained by the low colonization rate of inoculated seedlings at the beginning of the test. The higher effectiveness of indigenous AMF in increasing pinnule P content was not expected.

The lack of pH effect on shoot dry matter in both inoculated and not-inoculated soils was probably due to lack of pH effect on root colonization and pinnule P content. *A. koa* root dry matter was not affected by inoculation perhaps due to the low initial level of colonization by Leilehua and Wahiawa isolates.

Acacia koa shoot Al content was not affected by either pH change or inoculation. However, shoot Mn content significantly decreased with the increase in pH; probably due to the decrease of Mn availability at higher pH (Hue et al., 2001).

6.5.2. Mn-toxic soil

One of the effects on the symbiotic association with AMF in acid soils with Mn toxicity problem reported in literature is to increase the tolerance of plants to that stress. However in the current study, the lack of inoculation effect at low pH may have been due to the low initial level of inoculation of *A. koa* seedling before transplanting. This result implies that the quality and the quantity of the inoculum is crucial for the effectiveness of the symbiotic association.

The stimulation of pinnule P at low pH by the Leilehua isolates compared to the Wahiawa isolates and the indigenous AMF may be related to better resistance of the isolates to high soil Mn. However, at high pH the Wahiawa isolates performed better than the indigenous AMF and Leilehua isolates. These differences in pinnule P were not related to any difference in shoot dry mater or root dry matter.

The suppression of shoot dry matter with the decrease in pH can be explained by the high level of Mn (380 compared to 20 mg kg^{-1} at pH 4.5 and 6.6, respectively). Liming significantly decreased shoot Mn concentration. The lack of effect of isolates or indigenous AMF on shoot dry matter could be explained by the low level of colonization by isolates before transplanting. The high Mn content in shoots at low pH may be due to the higher Mn availability at that pH (Horst, 1988).

6.6. Conclusions

The result of our study did not allow us to determine the effectiveness of the isolates in protecting *A. koa* against Al and Mn toxicities because of the low colonization rate before transplanting. Further trials should be conducted with well colonized

seedlings in order to evaluate the effect of the different AMF isolates on *A. koa* growth in Al- and Mn-toxic soils

7. General conclusions

In summary these series of experiments led to the following conclusions:

1. Low soil pH coupled with Al or Mn toxicity seriously reduced *A. koa* growth. The toxic levels of Al and Mn for *A. koa* were estimated to be 0.64 $cmol_c$ Al kg^{-1} in Al-toxic soil and 92 mg Mn L^{-1} in Mn-toxic soil based on the 20% growth reduction. In our knowledge, this information was lacking in the literature.
2. *Glomus aggregatum* attenuated the effect of Al on *A. koa* root colonization, pinnule P content and biomass accumulation in Al-toxic soil. Whereas in Mn-toxic soil, inoculation with AMF had a positive effect on the growth only at pH 6.4.
3. The effectiveness of *G. aggregatum* in improving the growth of the two cultivars of *L. leucocephala* in acid soils depends on the genotypic difference. Contrary to the information in the literature, our study showed that cv K8 is less sensitive to soil acidic condition compared to cv K636.
4. Because of the low colonization of the seedling before transplanting, the effectiveness of the Leilehua and Wahiawa isolates could not be established in alleviating Al and Mn toxicities.

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