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Growth and development of jack-bean and pigeon-pea in cassiterirte mine spoil

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Cassiterite mining has caused critical damages to the environment with respect to soil degradation and water pollution. In a greenhouse experiment two leguminous species, jack-bean (*Canavalia ensiformis*) and pigeon-pea (*Cajanus cajan*), were grown using cassiterite mine spoil as substrate, in the presence of rhizobia and with or without mycorrhizal fungi, and the substrate was amended or not with organic compost and thermo-phosphate, to evaluate the potential development of these plants to grow on this substrate. Shoot height and shoot dry weight, nitrogen and phosphorus shoot contents, root nodule numbers and their dry weight were determined. Amendment with organic compost was important for all observed parameters in both species, but jack-bean had greater shoot N contents and biomass and less mycorrhizal dependence, when comparing with pigeon-pea. However, it was verified that efficiently nodulated plants, as pigeon-pea, can be fundamental for soil reclamation, by reducing the need for fertilizers and by stimulating the biological activity in soil. In our study the utilization of organic compost was crucial for plant growth, while thermophosphate was not always essential, especially in the presence of mycorrhiza. However, the mycorrhizae were essential, at least for pigeon-pea.

Key words: Cassiterite mine spoil, *Canavalia ensiformis*, *Cajanus cajan*, amazon region.

INTRODUCTION

The Amazon region is one of the largest but still little known or explored regions of the world with a potential for mineral exploration. Mining already is a fundamental economic feature of the region. In the 70´s and 80´s a gold run was responsible for its occupation by 800 thousand miners (Santos, 2002). For cassiterite, 92% of the mines are located in the Amazon region and are responsible for 10% of the world's production, while the State of Rondônia is the second greatest Brazilian producer (Porsani et al., 2004).

Nevertheless, mining activity always has a strong impact on the environment, with harmful consequences to plant and soil microbial communities (Pfleger et al., 1994). The establishment of plant species in mining areas is a challenge, mainly because of the withdrawal of the surface horizon or topsoil that comprises the main storage of nutrients, soil fauna and microorganisms (Ingram et al., 2005). Plants capable of establishing symbiosis with arbuscular mycorrhizal fungi (AMF) can be used to accelerate the recovery process of these areas (Zangaro et al., 2000).

As used in a Wyoming mine (Anderson et al., 2008), the transportation of topsoil for the reclamation of mining areas is also done in Brazil. However, quite often this topsoil has been buried below the mine spoil and cannot be recuperated. However, the use of some other practices, as grass sowing and mulching can reduce the impact and help in mine land reclamation, with a positive influence on the soil organic carbon and the microbial

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diversity (Mummey et al., 2002a). In the mulching process, fertilizer application may also be necessary to improve plant growth and establishment (Brofas et al., 2007). In some situations, the grass cover can help to increase the microbial biomass and soil organic carbon and act as a sink for sequestering carbon. Although, this is a typical reclamation practice, it may need years for the grass to promote conditions for the establishment of a more diverse plant community (Anderson et al., 2008).

The reclamation of open mining areas can only be considered accomplished after restoration of the soil functions, which involve the restoration of the microbial community and the biogeochemical cycles, fundamental for plant nutrition (Chodak et al., 2009; Ingram et al., 2005; Jordan et al., 2000).

Therefore, in land reclamation activities, certain microorganisms can be essential for the restoration of soil fertility and for facilitating the reestablishment of plants. Diazotrophic bacteria, especially rhizobia, capable of biological nitrogen fixation (BNF), and AMF are among these microbial tools. Nitrogen is a limiting nutrient for plant growth and the utilization of N fertilizers demands repeated applications, which is very expensive and can also cause damage to the environment if not used adequately (Franco and Faria, 1997). The symbiosis involving BNF and leguminous plants, however, does provide a continuous source of nitrogen since the yearly amount of biologically fixed N may reach 200 Tg N(Bockman, 1997).

The mycorrhizal symbiosis is based on changes between the partners in which the AMF help the host plant in phosphorus uptake, while the host plant provides carbon sources for the fungi (Jakobsen et al., 1994). This symbiosis is important for the sustainability of the tropical agro-ecosystems (Jordan et al., 2000), and is fundamental for the insertion of plants in degraded land areas, as in mine spoil (Lambert and Cole, 1980; Rao and Tak, 2001). This symbiosis can also contribute to the insertion of plants in heavy-metal contaminated soil by protecting the plant and increasing plant growth under stressing conditions (Soares and Siqueira, 2008). Legumes and herbaceous species, as jack-bean and pigeon-pea, are dually symbiotic with rhizobia and AMF and increase the carbon and nitrogen stocks in the soil (Diekow et al., 2005). Therefore, the tripartite symbiosis between legumes, rhizobia and AMF is an important alternative for the recovery of mine spoil degraded areas.

The reclamation of soils on previously mined lands takes more than 20 years to reach similar characteristics to those of an undisturbed soil with respect to microbial biomass and diversity (Chodak et al., 2009; Mummey et al., 2000b). Herbaceous plants, as jack-bean and pigeon pea, grow much faster when being part of the tripartite symbiosis, and can help in the establishment of native woody species and thus reduce the use of fertilizers (Rao and Tak, 2001).

The aim of this study was to evaluate the recuperation of degraded soils in areas left behind after cassiterite mining, by using organic compost and herbaceous legumes. The focus of this preliminary study was to demonstrate that jack-bean (*Canavalia ensiformis* (L.) DC) and pigeon-pea (*Cajanus cajan* (L.) Huth), in combination with rhizobia and AMF and in the presence of organic compost and thermo-phosphate amendment, can establish on cassiterite mine spoil in the greenhouse, and produce healthy and vigorous plants.

METHODOLOGY

The substrate used was cassiterite mine spoil, obtained from CESBRA-BRASCAN in the National Forest (FLONA) of Jamari, Rondônia State, in the Amazon region. The tropical forest covers 90% of the total area of 222.000 ha, in this FLONA. The chemical characteristics of the mine spoil were determined as follows: organic matter, by colorimetry; available phosphorus, by colorimetry; exchangeable potassium, by flame emission ph*otometry*; pH, by potentiometer (in 0.01 mol L^{-1} $CaCl_2$); exchangeable calcium, magnesium and aluminum were extracted with 0.1 mol L^{-1} KCl and analyzed by t*itulometry*; micronutrients as Cu, Fe, Mn and Zn were extracted with DTPA-EDTA and determined by plasma emission spectrometry, boron was extracted with 0.125% $BaCl_2.2H_2O$, and analyzed by colorimetry and SB (sum of exchangeable bases), $H+Al^{3+}$ (total acidity) by SMP buffer, T (sum of total cations), m% (aluminum saturation), V% (base saturation), $S\text{-}SO_4$ (sulfates), as described in Van et al. (2001). The microbial activity of the substrate was assessed by a basal and induced respirometric test (Alef, 1995).

The two leguminous species, jack-bean and pigeon-pea, were sown on the cassiterite mine spoil, after seed inoculation with recommended rhizobial strains: SEMIA-6156 and SEMIA 6158 (for jack-bean), and SEMIA-6156 and SEMIA 6157 (for pigeon pea). These rhizobia were multiplied in yeast mannitol broth (YMB) on a shaker for 8 days, according to Somasegaran and Hoben (1985). All plants of every treatment received rhizobia inoculation, and therefore the presence or absence of rhizobia was not one of the tested variables, since it is already well documented that the rhizobial symbiosis is of great value for the development of these leguminous plant.

The AM fungi used for inoculation were a mixture of *Glomus clarum*, *G. intraradices* and *Gigaspora rosea,* by applying about 30 g of a mixture of soil, spores, hyphae and colonized roots to each pot. The organic compost was obtained from the compost plant of the Waste Recycling Center of the University (CEPARA/ESALQ/USP, Brazil). The original ingredients were plant residues from the Campus and cow dung. This compost contained 1% nitrogen, 40% organic matter, 40% moisture and a C:N rate of 18:1 (dry weight). The compost was mixed with the mine spoil at a dose equivalent to 30 ton ha^{-1} (on a dry weight basis). The thermo-phosphate YOORIN (16.5% P_2O_5 soluble in 2% citric acid), was applied as a single dose, equivalent to 40 kg ha^{-1} of P_2O_5.

After 60 days, the plant height was measured and plants were harvested, and then roots were separated from the shoots. The shoot dry weight was determined after drying in an oven at 65°C. The nodules were removed, dried at 65°C, and the number of nodules and nodule weight were determined.

The shoot nitrogen content was determined by sulphuric digestion followed by the semi-micro-Kjeldahl method. The phosphorus content was determined by nitric-*perchloric* digestion and estimated by metavanadate colorimetry (Malavolta et al., 1989). After harvesting of plants, the soil pH in 0.01mol/L $CaCl_2$ was also determined.

The experimental design was completely randomized, with a factorial arrangement 2 (with and without AMF) x 2 (with and

Table 1. Chemical characteristics of cassiterite mine spoil used as substrate for the growth of pigeon pea and jack-bean.

				mmol dm^{-3}									Micronutrients (mg dm^{-3})				
pH($CaCl_2$)	OM(g dm^{-3})	P(mg dm^{-3})	S-SO_4(mg dm^{-3})	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al^{3+}	SB	T	V(%)	m (%)	B	Cu	Fe	Mn	Zn
4.8	9.0	2.0	60.0	0.1	1.0	1.0	1.0	13.0	2.1	15.1	14	32	0.12	0.1	6.0	2.0	0.2

Legend: OM = organic matter, SB= sum of exchangeable bases, T= sum of total cations, m% = aluminum saturation, V% = base saturation.

Table 2. Effect of the interaction (F test, $p < 0.05$) between mycorrhiza and organic compost and mycorrhiza and thermo-phosphate on shoot height, shoot dry matter, shoot nitrogen content, shoot phosphorus content and nodule dry matter of *C. cajan* grown on mine spoil in the greenhouse (n=4). Treatments followed by the same letter for each variable do not differ by the *t* test ($p < 0.05$).

	Mycorrhiza and organic compost interaction			
Variables	**Control**	**Mycorrhiza**	**Organic compost**	**Mycorrhiza and organic compost**
Shoot height (cm)	12.91^c	15.38^c	28.84^b	38.42^a
Shoot dry matter (g)	0.33^d	0.57^c	1.22^b	2.73^a
Shoot nitrogen content (mg $plant^{-1}$)	6.85^c	10.82^c	$21,60^b$	64.93^a
Shoot phosphorus content (mg $plant^{-1}$)	2.17^c	3.82^c	8.22^b	19.55^a
Nodule dry matter (mg $plant^{-1}$)	22.5^c	32.5^c	$56.\ 3^b$	14.12^a
	Mycorrhiza and thermo-phosphate interaction			
Variables	**Control**	**Mycorrhiza**	**Thermo-phosphate**	**Mycorrhiza and thermo-phosphate**
Shoot dry matter (g)	0.77^c	1.50^b	0.78^c	1.79^a
Shoot phosphorus content (mg $plant^{-1}$)	5.22^c	10.51^b	5.17^c	12.86^a
Nodule dry matter (mg $plant^{-1}$)	40.0^c	77.5^b	38.8^c	96.3^a

without thermophosphate) x 2 (with and without organic compost), with 4 repetitions. The data expressed as percentage were transformed using $(x/100)^{1/2}$ and the data originated from counts, using $(x + 0.5)^{1/2}$. The data were submitted to analysis of variance (ANOVA) and the means were compared using Student's *t* test at 5 % significance (SAS Institute Inc.).

RESULTS

The chemical analysis of the cassiterite mine spoil showed a very low-fertility substrate (Table 1). CO_2 evolution was extremely low (0.08 mg g^{-1} day^{-1} of CO_2), even in the presence of glucose (0.12 mg g^{-1} day^{-1} of CO_2), what demonstrates that this residue is almost sterile.

The organic compost amendment and the interaction with AMF stimulated the growth of pigeon-pea, increasing plant height and shoot dry matter (*t* test, $p < 0.05$) (Table 2). Thermo-phosphate was effective in increasing shoot dry matter only in the presence of AMF.

In pigeon-pea, organic compost amendment had a significant effect on shoot nitrogen content only in the presence of AMF (Table 2). Phosphorus content showed a positive response to thermo-phosphate and organic compost, but only when inoculated with AMF (Table 2). The presence of AMF was essential to promote the increase in nodule dry matter when amended with thermo-phosphate and this value surpassed nodule dry weight of plants that had received only AMF or only thermo-phosphate (Table 2). The organic compost amendment, alone or in the presence of AMF, was essential to promote the increment of the nodule dry matter in pigeon-pea

(Table 2).

In pigeon-pea the number of nodules increased 28% in the presence of organic compost, while AMF and thermo-phosphate alone had no significant effect. However, in the presence of AMF, the thermo-phosphate doubled the nodule dry matter, and the organic compost produced an increase of 7.4 times over the control (data not shown). For the root percent colonization there was interaction only between AMF and organic compost amendment (F test, $p < 0.05$). Root percent colonization was greater when the plant received only AMF inoculation showing a rate close to 34% and decreased to 27% when in the presence of organic compost. However, a moderate mycorrhizal dependence of 48% was observed.

Only organic compost amendment influenced jack-bean shoot height, shoot dry matter, phosphorus and nitrogen content and the nodule dry matter (F test, $p < 0.05$). The shoot height increased from 11.9 to 17.1 cm; the shoot dry matter increased from 2.88 to 5.45 g. The shoot phosphorus and the nitrogen contents increased from 7.94 to 10.06 mg/plant and from 68.2 to 126 mg/plant, respectively. The nodule dry matter increased from 0.12 to 0.25 g/plant, when compared with that of the plants that had received no organic compost amendment. However, only AMF and organic compost influenced the number of nodules separately. Thus, the organic compost increased the mean value of the treatments that received AMF from 8.25 to 9.85 nodules per plant and from 7.49 to 10.6 nodules,

The mycorrhizal root colonization was greater when the plant received organic compost amendment, showing a rate close to 33% and decreased to 20%, when grown only with AMF or in the presence of thermo-phosphate (data not shown). Thermo-phosphate, in the presence of AMF, also produced a greater colonization index, 23.0% against 16% for AMF inoculation alone (data not shown). However, the mycorrhizal dependence was close to zero.

DISCUSSION

The very low CO_2 values evolved as well in basal as in induced respiration from the mine spoil indicated that few microbes had been able to survive in this substrate. Chodak et al. (2009) also found low microbial activity in soil when testing the degradation of different carbon substrates by the Biolog® methodology in a chronosequence of sandy mine soils, in Poland, a result that characterizes low functional diversity. Similarly, in our case, the very low respiration indices demonstrate the necessity of a microbial enrichment in this degraded mine soil. The most important factors that stimulate plant growth and improve the reforestation of degraded areas are nitrogen and phosphorus, besides water. When cultivated with organic compost and colonized by mycorrhizal fungi, pigeon-pea showed the highest dry matter, height, phosphorus, nitrogen shoot content and nodule dry matter. The application of thermophosphate was also important for the shoot dry matter, nodule dry matter and phosphorus content, when in the presence of AMF.

The incorporation of organic compost into degraded or semi-arid areas can improve the chemical characteristics of the soil due to its relative stability and promote plant densification in the soil cover (Ros et al., 2003), by enhancing microbial activity, modifying the rhizosphere and promoting sustainable growth, thereby improving the biogeochemical cycles (Ingram et al., 2005; Park et al., 2010). Chodak and Niklińska (2010) also found that soil texture and fast growing tree species as birch and pines, are important to modify the organic carbon and microbial biomass content. Furthermore, as described in Brofas et al. (2007), faster growing species, as *Lolium multilflorum* and *Phacelia tanacetifolia*, can help in preventing soil erosion. This management can also inhibit the insertion of other species in the beginning; later on the reduction of plant density, however, will allow for the invasion of native species.

The shoot N and P concentrations for pigeon pea were highest in treatments with AMF and organic compost. AMF and organic compost were also important to promote the nodule dry matter in pigeon pea. AMF can interact with nitrogen fixing bacteria, stimulating nitrogen uptake (Rao and Tak, 2001). This growth improvement is also important to increase soil nutrients through litter deposition, decomposition and humus formation (Berg and Matzner, 1997). Several authors showed that the development of plants inoculated with mycorrhizal fungi significantly increased in the presence of organic compost (Gaur and Adholeya, 2002), as observed for pigeon pea.

Jack-bean presented the higher shoot nitrogen content, nodule number and weight, in the presence of organic compost, when comparing with pigeon pea. Therefore, for the recuperation of mine areas the utilization of these two species may be relevant, especially when in the presence of organic compost. Ae et al. (1990) found that pigeon pea could increase the phosphorus availability in P-deficient cropping systems by means of root exudates when the plants were mycorrhizal and presented a higher growth rate. Pigeon pea seems to be a good alternative for mining areas, by making the phosphorus available for other plants in the system. Once a legume is established, the lack of phosphorus is the limiting factor for fixing atmospheric nitrogen in mine spoil substrates. Thus, legumes with a better scavenging ability for phosphorus will fix more N for their nutritional needs (Franco and Faria, 1997). Jack-bean can be important to increment the nitrogen and phosphorus content and is independent of AMF inoculation, except for nodule weight.

AMF-inoculated plants generally present a greater tolerance to transplanting and water stresses, resistance to pathogens, and faster growth rates than non-inoculated plants (Jordan et al., 2000; Pfleger et al., 1994).

Increasing the organic carbon and soil microbial biomass can stimulate the catabolic diversity of soil microorganisms and this fact can improve the stability of the soil microbial communities under stress or disturbing situations (Degens et al., 2001). The initial plant cover, especially mycorrhizal plants, can also enable the introduction or the reintroduction of native species (Allen, 1989; Barea et al., 2002).

Since the thermo-phosphate was applied in a small amount, it is plausible to infer that this dose was not high enough for effective plant growth, since the content of organic matter was also very low in the mine spoil (Longo et al., 2005), as a consequence of the withdrawal of the soil litter and an accelerated decomposition rate of the remaining organic matter, after the removal of the A horizon during mining (Ingram et al., 2005). Thus, for the early stages of re-vegetation, the organic compost is an essential factor to support the establishment of a sustainable biota and plant cover.

Conclusion

We could show that under stressing conditions maximum plant growth was only achieved when at least two of the analyzed variables were present together acting on plant development; thus, these interactions among plants, microbes and fertilizers promote a sustainable and balanced growth system.

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