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Arbuscular mycorrhizal fungi in a semiarid copper mining area in Brazil

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Abstract The occurrence of arbuscular mycorrhizal fungi (AMF) in a copper mining area was investigated. Soil samples were collected from six sites at the Mineração Caraiba, Bahia State, northeastern Brazil, comprising: (1) a site that receives the waste product; (2) a site that receives low grade deposits; (3) the interface between the caatinga and site 1; (4) the surroundings of the industrial area; (5) the site for extracting topsoil for land filling; (6) the preserved caatinga. Thirty-two plant species were identified around the collection locations. Trap cultures were maintained in the greenhouse for 3 months, using bahia grass (*Paspalum notatum* Flügge) as the host plant. Spores were extracted from soil and 21 AMF species (15 *Glomus* and one of each of *Acaulospora, Archaeospora, Entrophospora*, *Gigaspora*, *Paraglomus* and *Scutellospora*) were identified. In site 1, plants or AMF were not found during the dry season. Site 6, with native vegetation, had the highest number of plants and AMF species. The disturbed sites showed less plant diversification, with the community of AMF being quantitative and qualitatively affected by disturbance.

Keywords Arbuscular mycorrhiza · Heavy metals · Glomeromycota · Disturbed area · Caatinga

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Introduction

There are >160 species of arbuscular mycorrhizal fungi (AMF), whose taxonomic history is recent. Based on their symbiotic habit and morphology, Morton and Benny (1990) included all of the AMF in the order Glomales, with three families (Acaulosporaceae, Gigasporaceae and Glomaceae) and six genera (*Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*). However, Redecker et al. (2000), using phylogenetic information obtained from morphological and molecular data, transferred the species of *Sclerocystis* to *Glomus*, reinforcing the proposal of Almeida and Schenck (1990). Morton and Redecker (2001) created two new families, Archaeosporaceae and Paraglomaceae, respectively, typified by the genera *Archaeospora* and *Paraglomus*, and currently the AMF are included in a new phylum: Glomeromycota (Schüssler et al. 2001).

The distribution of AMF in natural Brazilian ecosystems has not been well studied: Trufem (1996) mentioned some species in the Amazon and Atlantic provinces, as well as in cerrado areas, without records for other important areas (caatinga in the northeast and pampas in the south). Taxonomic inventories of AMF in undisturbed areas were done in São Paulo (Bononi and Trufem 1983; Trufem 1988, 1990; Trufem et al. 1994; Gomes and Trufem 1998) and Santa Catarina (Stürmer and Bellei 1994). In cultivated areas there are records for the States of São Paulo (Trufem and Bononi 1985; Trufem et al. 1989, 1990; Grandi and Trufem 1991; Carrenho et al. 2001) and Pernambuco (Maia and Trufem 1990; Melo et al. 1997).

The excess of heavy metals in soils has a direct toxic effect on plants, being deleterious to the AMF and having an impact on plant and microbial communities (Valsecchi et al. 1995). Various heavy metals are fungitoxic, reducing spore germination, mycelial growth and, consequently, mycorrhizal colonization (Nogueira 1996). An excess of Zn and Cu inhibits spore germination (Hepper 1979), while colonization can be reduced in the presence of high levels of Zn, Cu, Ni, and Cd (Gildon and Tinker 1983).

There are apparently no papers that mention species of AMF in areas in Brazil that have been degraded by mining. However, taxonomic surveys in these areas are important to provide information regarding environmental impact and also about the AMF species that are adapted to this stress condition, and would be useful for revegetation programs.

This paper deals with the identification of AMF species that occur in areas affected by copper mining, relating the presence of such species to the levels of environmental impact found in the areas and comparing the occurrence of these fungi with the local plant diversity.

Materials and methods

Study area

The Caraíba Copper Mine is situated in Pilar, Jaguararí Municipality, Bahia State (9°51′43″S, 39°53′50″W; Fig. 1). The area is mainly flat with emerging rocks, has an annual precipitation of 400 mm, with the rainy season mostly from November to April, and an average temperature of 25°C. The climate is tropical semiarid, the soils are sand-clay and clay types and the vegetation is a typical caatinga (information from Mineração Caraíba). The caatinga is a kind of dry forest, characterized by trees and shrubs which are deciduous during the dry season. Many of these plants are thorny, while some are succulent and leafless (Cactaceae and some Euphorbiaceae). There are also many Bromeliaceae and some herbs, mostly annual (Andrade-Lima 1964; Sampaio 1995).

Soil samples were collected in the dry season (August 1998), and in the rainy season (February 1999), from six sites (Fig. 1). Of these, five showed environmental disturbance produced by mining activity. One was preserved caatinga, and was considered as the control site. In each site seven samples of approximately 2.5 kg soil (extracted from the 5–20 cm depth) were taken, preferably from the rhizosphere of plants, with the sampling places being selected at random. Within a 2-m radius at each sampling place the plant species (trees/shrubs/herbs) were recorded. The plants found near the sampling place were identified at the [Centro de Pesquisa do Trópico Semi-árido-Empresa Brasileira de Pesquisa Agropecuária (Cpatsa-Embrapa)]. Soil was analysed using Mehlich's method for available P, K, Cu, Zn, Fe and Mn (HCl 0.05 mol l^{-1}+H_2SO_4 0.025 mol l^{-1}) and for Al, Ca and Mg (KCl 1 mol l^{-1}), both described in Embrapa (1997) (Table 1).

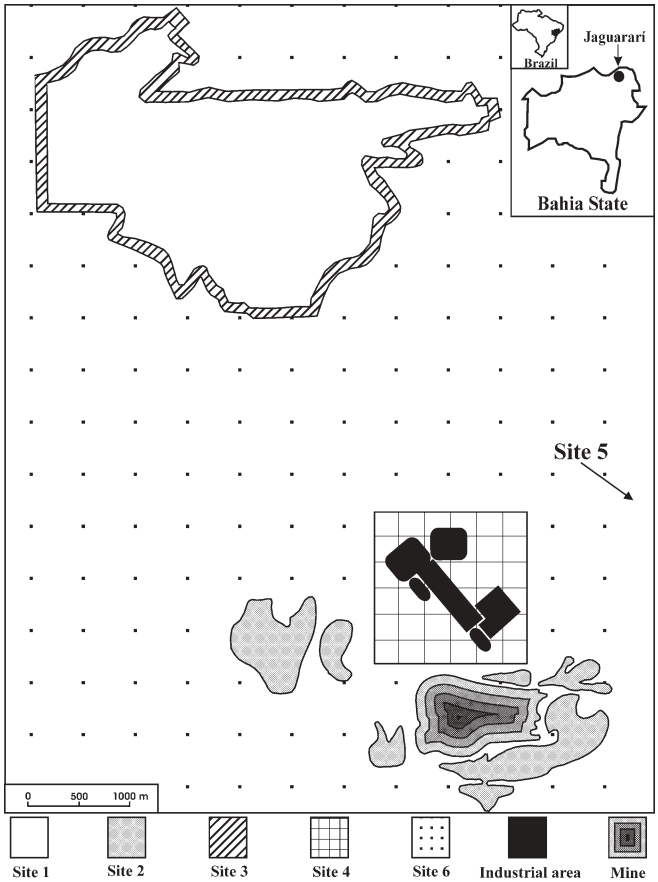

Fig. 1 Collecting sites in the Caraiba copper mine, Jaguararí, Bahia State (*upper right inset*). *1* Site that receives the waste product, *2* site that receives the lowgrade deposits, *3* the interface between the caatinga and the waste product area, *4* the surroundings of the industrial area, *5* the site for extracting topsoil for the purpose of land filling, *6* the preserved caatinga

Characterization of sites

Site 1

This site contains the accumulated waste formed by rock powder from the industrial extraction of Cu in the crushed ore. It occupies 580 ha, and seems to be a desert: there are hardly any plants, except for the few usually found only during the wet season.

Table 1 Chemical and physical analysis of soil from sites of the Mineração Caraíba, Jaguarari, Bahia State. *1* Site that receives the waste product, *2* site that receives the lowgrade deposits, *3* interface between the caatinga and site 1, *4* surroundings of the industrial area, *5* site for extracting topsoil for the purpose of land filling, *6* preserved caatinga; data are averages of seven collecting points for each site except site 1

Sites	P	Cu	Mn	Zn	Fe	K	Mg	Ca	Al	pH (H_2O)	Particle Size		
	(mg dm^{-3})					($cmol_c$ dm^{-3})				1:2.5	Sand	Silt	Clay
1	266	433.4	53.4	5.7	434.4	0.06	1.4	5.2	0.00	8.2	93	3	4
2	167	606.7	44.0	5.2	168.1	0.27	5.9	7.1	0.01	7.3	69	21	10
3	179	445.4	47.8	5.0	337.1	0.61	1.8	6.0	0.00	7.8	61	35	4
4	44	86.2	33.4	2.0	65.9	0.25	5.9	10.5	0.04	6.9	60	24	16
5	95	30.4	48.6	1.6	52.4	0.22	5.4	9.4	0.17	7.1	65	19	16
6	141	2.7	46.4	2.2	33.6	0.30	3.7	8.0	0.05	6.2	62	24	14

Table 2 Species of plants present within a 2-m radius at each sampling place at the Mineração Caraíba, Jaguarari, Bahia State. For a description of sites, see Table 1

Family	Common name (in Brazil)	Scientific name	Sites					
			1[a]	2	3	4	5	6
Anacardiaceae	Aroeira	*Myracrodruon urundeuva* Allemao			X			
	Baraúna	*Schinopsis brasiliensis* Engl.						X
	Umbuzeiro	*Spondias tuberosa* Arruda			X			X
Apocinaceae	Pereiro	*Aspidosperma pyrifolium* Mart.			X			
Boraginaceae	Moleque duro	*Cordia leucocephalla* Moricand.						X
Bromeliaceae	Caroá	*Neoglaziovia variegata* (Arruda) Mez.			X			X
	Macambira de flecha	*Eucholirium spectabile* Mart. ex Schult.f.						X
Burseraceae	Umburana	*Commiphora leptophloeos* (Mart.) J.B. Gillet.						X
Cactaceae	Palmatória	*Opuntia palmadora* Br. et R.						X
	Mandacarú	*Cereus jamaracu* DC.				X		
	Xique-xique	*Pilosocereus gounellei* (Weber) Byl. et Rowl			X	X		X
Caesalpinaceae	Catingueira	*Caesalpinia pyramidalis* Tul.						X
Capparaceae	–	*Capparis yco* Mart. ex Eichl						X
	Muçambê	*Cleome spinosa* Jacq.	X				X	
Euphorbiaceae	Pinhão	*Jatropha molissima* Baill.	X		X	X	X	X
	Cansanção	*Cnidosculus urens* (L.) Arthur			X			X
	Favela	*Cnidosculus phyllacanthus* Pax et K. Hoffman			X	X		X
	Maniçoba	*Manihot pseudoglaziovii* Pax. et K. Hoffman.						X
	Marmeleiro-preto	*Croton sonderianus* Müll. Arg.						X
	Pinhão mirim	*Jatropha ribifolia* (Pohl) Baill.					X	
	Velame	*Croton campestris* (St. Hil.) Müll. Arg.				X		X
Malvaceae	Malva	*Gaya* sp.	X		X	X	X	X
	Malva branca	*Sida cordifolia* L.					X	
	Malva de sebo	*Herissantia tiubae* (K. Schum.) Briz.					X	
Mimosaceae	Algaroba	*Prosopis juliflora* (SW.) DC.		X		X	X	
	Angico	*Anadenanthera macrocarpa* (Benth.) Brenan						X
	Jurema-vermelha	*Mimosa arenosa* (Willd.) Poir.			X			
	–	*Mimosa* sp.						X
	Carqueja	*Calliandra depauperata* Benth.			X			
Polygonaceae	Pau caixão	*Ruprechtia apetala* Wedd.			X			X
Solanaceae	Fumo bravo	*Nicotiana glauca* Graham	X	X	X		X	
Sterculiaceae	Imbira vermelha	*Melochia tomentosa* L.			X	X	X	X
Total			4	2	14	8	9	21

[a] Plants only found during the second sampling (from Silva et al.2001)

Site 2

This is an area with low-grade deposits in the form of mining waste (piles of rock) with low amounts of copper (economically not viable for industrial extraction). These piles, that are almost 20 m high in some places and occupy approximately 240 ha, received a thin layer of soil from site 5. In this place some specimens of *Prosopis juliflora* (Sw.) DC. and *Nicotiana glauca* Graham, with a scattered distribution, were observed.

Site 3

This site forms the interface between sites 1 and 6, and is a place where dead plants and dry plants, without leaves, were found. This site had a layer of waste of 20–50 cm on top of the natural soil.

Site 4

This site included the surroundings of the industrial area, and is where the ore is processed. It is characterized by the intensive transit of vehicles, dust, mining residues and industrial waste. It has some native caatinga species together with some introduced ones, such as *P. juliflora*.

Site 5

In this site topsoil is extracted for land filling and a superficial layer of soil was taken to cover the piles of rock in site 2. Some invasive shrub plants are found here. An attempt to revegetate this area with seedlings of tree species was made, without success. It has the same invasive herbaceous species as the remainder of the area.

Site 6

This site is a preserved caatinga with limited alteration of the native vegetation found in the region.

Identification of AMF

For each soil sample, trap cultures were prepared to allow multiplication of the AMF spores. The cultures were maintained in the greenhouse, using *Paspalum notatum* Flügge as the host. After 3 months, AMF spores were extracted from the soil by wet sieving (Gerdemann and Nicolson 1963), and sucrose centrifugation (Jenkins 1964), mounted in polyvinyl alcohol lactoglycerol and observed with a light microscope. Species were identified using Schenck and Pérez (1990) and new descriptions found in Morton (2001).

The index of similarity between AMF and plant species in the areas was evaluated (Sorensen 1978).

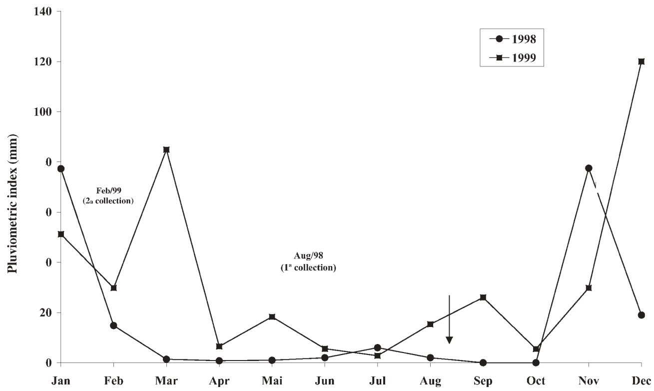

Fig. 2 Pluviometric index from data registered at the Mineração Caraíba, Jaguarari, Bahia State (source: Mineração Caraíba). *Jan* January, *Feb* February, *Mar* March, *Apr* April, *Mai* May, *Jun* June, *Jul* July, *Aug* August, *Sep* September, *Oct* October, *Nov* November, *Dec* December

Results

Thirty-two plant species belonging to 14 families and 28 genera were identified (Table 2). The site with preserved caatinga (site 6) had the highest number of species (21). Sites 3, 5 and 4 had respectively 14, 9, and eight species. These values represent a >33% reduction in numbers of plant species in relation to site 6.

Sites 1 and 2, with waste material deposits, had a reduced number of species, approximately <20% of the number of species recorded at the preserved site. The exotic species (*P. juliflora*) occurred abundantly in the sites affected by mining, except in site 1. Native species such as *Nicotiana glauca*, *Gaya* sp., *Jatropha molissima,* and *Cleome spinosa* can apparently tolerate the stress produced by mining in the most affected sites.

Twenty-one species of AMF were identified, most of them belonging to *Glomus* (Table 3). The only *Acaulospora* species was found in sites 3, 4 and 6. Species of *Archaeospora*, *Entrophospora*, *Gigaspora,* and *Scutellospora* were observed only in site 6. *Glomus macrocarpum* was the only species identified in all sites, while *G. etunicatum*, *G. microcarpum*, *G. sinuosum,* and *P. occultum* were isolated from five of the six sites.

Site 6 (with native vegetation) showed the highest number of AMF species (15) followed by sites 2 and 5 (11 taxa), and 3 and 4 (9 taxa). In site 1, only one species was found, during the rainy season.

The months before the first collection were characterized by very low rainfall, compared with those before the second collection (Fig. 2). Some AMF species were isolated in both seasons, but many occurred only in the dry or rainy season. In each site there was variation in the composition of AMF species between the collecting

Table 3 Species of arbuscular mycorrhizal fungi (*AMF*) in sites of the Mineração Caraíba, Jaguarari, Bahia State. *S* Species found only during the dry season, *C* species found only during the wet season, *A* species found during both seasons; for a description of sites, see Table 1

AMF	Sites					
	1	2	3	4	5	6
Acaulospora scrobiculata Trappe			C	S		S
Archaeospora leptoticha (Schenck & Smith) Morton & Redecker						S
Entrophospora infrequens (Hall) Ames & Schneider						A
Gigaspora margarita Becker & Hall						A
Glomus albidum Walker & Rhodes						C
G. clarum Nicol. & Schenck		S			S	
G. diaphanum Morton & Walker				C		C
G. etunicatum Becker & Gerdemann		C	A	A	A	A
G. invermaium Hall		C			S	
G. macrocarpum Tulasne & Tulasne	C	C	A	A	A	A
G. microaggregatum Koske, Gemma & Olexia						S
G. microcarpum Tulasne & Tulasne		C	A	S	A	A
G. mosseae (Nicol. & Gerd.) Gerdemann & Trappe			S	A	A	A
G. sinuosum (Gerd. & Baski) Almeida & Schenck		S	A	A	A	A
G. tortuosum Schenck & Smith		C	S			
Glomus sp. 1		S		S	S	
Glomus sp. 2		S			S	C
Glomus sp. 3		C			S	
Glomus sp. 4			C			
Paraglomus occultum (Walker) Morton & Redecker		A	A	A	A	A
Scutellospora gilmorei (Trappe & Herd.) Walker & Sanders						C
Total	1	11	9	9	11	15

Table 4 Similarity (Sorensen index) of plants and AMF between the sites of the Mineração Caraíba, Jaguarari, Bahia State. *P* Plants, *F* fungi; for a description of sites, see Table 1

Sites	1	2		3		4		5		6	
		P	F	P	F	P	F	P	F	P	F
1	100	33	17	33	22	33	20	62	17	16	13
2		100		13	63	20	60	36	91	0	46
3				100		45	71	35	53	51	52
4						100		47	70	41	67
5								100		20	54
6										100	

periods. In sites 1, 2 and 6, a higher diversity of AMF occurred in the rainy season, with the opposite recorded for sites 4 and 5. Site 3 (the interface) had the same number of AMF species in both seasons.

The highest levels of similarity between plants were found between sites 1 and 5 (62%) and sites 3 and 6 (51%) (Table 4). For the AMF, the lowest index of similarity was between sites 1 and 6, as only *G. macrocarpum* was found in the former (Table 4). Conversely, site 4 revealed a high similarity of AMF with the other sites (60–71%). The highest similarity of AMF occurred between sites 2 and 5 (91%).

Discussion

The diversity of plant and AMF species was reduced by the mining activities, with the decrease in plant diversity stronger than that observed for the AMF. A lower diversity of plants occurred in sites 1 and 2, both with a high amount of waste deposited on the surface and totally covering the native vegetation. During the dry season, species of plants or AMF were not observed in site 1, which suggests a high degree of impact in this place (Tables 2, 3).

The waste material in site 1 had an adverse effect on the plants, considering that a layer of this material covering the caatinga (site 3) was enough to reduce by >30% the number of plant species in relation to the native caatinga. A reduction of the phytotoxic effect of this waste may be possible with the addition of organic matter (O.J. Saggin-Junior, personal communication) favouring the establishment of the mycorrhizal symbiosis.

The diversity of detected AMF species can be compared with that recorded by Stutz et al. (2000), using trap cultures, in soils from arid and semiarid regions of the USA and Namibia. Some authors suggest that trap cultures may select the species that sporulate easily, leaving out those that, although not sporulating, colonize the roots (Turnau et al. 2001). However, for the identification of AMF using morphological characters, the use of trap cultures is recommended, because a large number of health spores are needed for correct identification. Spores collected directly from the field usually are dirty and do not maintain intact structures that are useful for identification, such as subtending hypha, sporiferous saccule, and others (Morton 2003).

More than 70% of the species found in the area belong to *Glomus* (15); species of this genus predominate in areas polluted by heavy metals (Pawlowska et al. 2000; Turnau et al. 2001) which indicates a tolerance to heavy metals. *Paraglomus occultum*, *G. etunicatum*, *G. microcarpum*, *G. sinuosum* and particularly *G. macrocarpum*, which occurred in all of the sites, should be tested in relation to their potential for use as an inoculum in revegetation programs in these areas affected by copper mining.

This constitutes the first record of *G. clarum* and *G. microcarpum* for the northeastern region, while *G. tortuosum* is recorded for the first time in Brazil. Four taxa of *Glomus,* with characters that do not relate to the descriptions available, probably constitute new species.

Among the native plant species, *J. molissima* and *N. glauca* were the only shrubs which apparently could tolerate the soil toxicity of the mining area and because of that may have potential for revegetation. In the absence of native species which are aggressive and stress tolerant, one should look for exotic species to initiate the establishment of vegetation in the area (Souza and Silva 1996). *P. juliflora* has such a potential, considering its distribution in the studied area. Leguminosae species such as *Mimosa caesalpinifolia* Benth. (native of the caatinga) and *Leucaena leucocephala* (Lam.) De Wit have been indicated in revegetation programs due to their production of plant matter: 5.8 and 10 t ha^{-1} $year^{-1}$, respectively (Dias et al. 1995).

The presence of only one AMF species in site 1 during the rainy season, and the absence of AMF during the dry season, is possibly due to the absence of plants, the excess of metals and high pH (8.2) of the soil. Apparently, the pH in some of the areas became higher with the deposition of waste (Table 1), which makes it difficult to establish native species of AMF in soils of caatinga that usually have an acidic pH.

According to Schenck and Siqueira (1987), *Glomus mosseae* prefers neutral or slightly alkaline pH. This species was observed in sites where the pH varied from 6.2 to 7.8. In areas with pH >7.0, species of *Glomus, P. occultum*, and *A. scrobiculata* were found, the latter in soil with pH 7.8. Maia and Trufem (1990) mentioned the possible "preference" of *Gigaspora* and *Entrophospora* for soils with acid pH, while *Glomus* and *Acaulospora* tolerate a wider range of pH. The data here presented are in accordance with those from Zambolim and Siqueira (1985) who mentioned that species of *Glomus* are the most common in neutral and alkaline soils.

The index of similarity showed the impact caused by the mining activity on the composition of plant and AMF communities, when comparing the diversity of the preserved caatinga (site 6) with those of the other sites, mainly with site 1 for the AMF, and sites 1 and 2 for the plants.

Silva et al. (2001) found a small number of infective propagules in the most impacted sites of the mining area, besides a negative correlation between the number of AMF spores and levels of Cu, Fe, and P, which indicates that high levels of these elements adversely affect the propagules of these fungi. The index of similarity between the vegetation communities of sites 1 and 5 was high (62%) in relation to the other sites, possibly because of the presence in both of invader plants which primarily colonize disturbed places. The high similarity between species of AMF found in site 4 in relation to the other sites is probably due to the transitional character of this site (i.e. contaminated by the wastes), where part is degraded by soil removal, and part still has native vegetation, although modified in terms of species composition. The similarity (91%) between the populations of AMF of sites 2 and 5 may be due to the fact that the soil from site 5 had been deposited over site 2. Many authors have mentioned the negative effect of mining and the presence of heavy metals in soils on AMF and plants (Hepper 1979; Gildon and Tinker 1983; Griffioen et al. 1994; Valsecchi et al. 1995; Nogueira 1996; Del Val et al. 1999). Studies in areas degraded by mineral extraction showed that the AMF were reduced or totally eliminated from these areas (Allen 1991). One of the causes is the contamination of soils by heavy metals that reduce plant growth and mycorrhizal colonization (Siqueira et al. 1999). In summary, the impact produced by copper mining reduced the community of AMF, depending on the level of disturbance. The reduction of plant diversity, the high pH and the amount of metals in the soil, possibly were the most important impact factors. Species of *Glomus* are the most common in semiarid ecosystems (Stutz et al. 2000), and those that show a wide tolerance to the edaphic modifications caused by mining constitute potential sources for the production of inoculum in revegetation programs.

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