FIELD ASSESSMENT OF MEAT AND BONE MEAL FOR MANAGEMENT OF GUAVA ORCHARDS AFFECTED BY GUAVA DECLINE

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ABSTRACT

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Guava decline is a disease complex involving *Meloidogyne enterolobii* and *Fusarium solani*, which causes root rot, defoliation and death of guava trees within months from onset of symptoms. Since no resistant cultivar or rootstock is available nor are there nematicides registered for this crop, management strategies for this disease are needed. Meat and bone meal (MBM) was applied to the soil of a commercial guava orchard affected by guava decline, in three dosage/regime applications: i) quarterly applications of 12.5 Kg/tree, ii) quarterly applications of 25 Kg/tree, and iii) semiannual applications of 50 Kg/tree. During this 24 month-experiment, the following variables were assessed: *M. enterolobii* and other soil plant-parasitic and free-living nematodes presence and population density, *Fusarium* sp. density in soil and guava roots, release of ammonia in the soil upon MBM decomposition, and productivity in two harvests. The quarterly application of 25 Kg/tree was further assessed in three different orchards, which were clearly distinct in age and in the levels of disease severity and agronomic care. The low levels of ammonia observed in the soil upon MBM decomposition in all three dosage/regime applications likely explain the modest reduction of second-stage juveniles of *M. enterolobii* in soil and other minor plant-parasitic nematodes. The parasitism by *M. enterolobii*, expressed as density of root galls, and density of *Fusarium* sp. in soil and roots were unaffected by MBM applications, which led to progression of guava decline and reduced the orchard productivity. This work shows the difficulty in management of plant-parasitic nematodes when organic amendments are applied in soil, particularly in the control of disease complexes as guava decline.

Key words: disease complex, *Fusarium solani*, *Meloidogyne enterolobii*, organic soil amendment, *Psidium guajava*

RESUMEN

Almeida, A. M., R. M. Souza, V. M. Gomes, T. F. Ferreira, and V. Mussi-Dias. 2013. Evaluación a campo de harina de carne y hueso para el manejo de plantaciones afectadas por el deterioro del guayabo. Nematropica 43:247-253.

El deterioro del guayabo es un complejo de enfermedad que involucra a *Meloidogyne enterolobii* y *Fusarium solani*; provoca pudrición radical, defoliación y muerte de los árboles a meses del inicio de los síntomas. Debido a que no hay disponibilidad de cultivares ni patrones resistentes o nematicidas registrados para este cultivo, son necesarias estrategias de manejo para esta enfermedad. En una plantación comercial afectada por el deterioro del guayabo, se aplicó harina de carne y hueso (HCH) al suelo en tres dosis/régimen de aplicación: i) aplicaciones trimestrales de 12.5 Kg/árbol, ii) aplicaciones trimestrales de 25 Kg/árbol, iii) aplicaciones semestrales de 50 Kg/árbol. Durante el experimento de 24 meses, se evaluaron las siguientes variables: presencia y densidad de población de *M. enterolobii*, otros nematodos fitoparásitos y de vida libre, densidad de *Fusarium* sp. en suelo y raíces de guayabo, liberación de amonio en el suelo después de la descomposición de HCH y la productividad en dos cosechas. La aplicación trimestral de 25 Kg/árbol se evaluó, además, en tres plantaciones con claras diferencias de edad, nivel de severidad de la enfermedad y cuidado agronómico. Los bajos niveles de amonio observados en suelo después de la descomposición de HCH en todas las dosis/régimen de aplicación, probablemente expliquen la escasa reducción de juveniles de segundo estadio de *M. enterolobii* y de otros nematodos fitoparásitos. El parasitismo de *M. enterolobii*, expresado como densidad de agallas en raíces, y la densidad de *Fusarium* sp. en suelo y raíces no fueron afectados por las aplicaciones de HCH; esto condujo a la progresión del deterioro del guayabo y redujo la productividad de la plantación. Este trabajo muestra la dificultad en el manejo de nematodos fitoparásitos cuando se aplican enmiendas orgánicas en el suelo, particularmente en el control de complejos de enfermedades como el deterioro del guayabo.

Palabras clave: complejo de enfermedad, *Fusarium solani*, *Meloidogyne enterolobii*, enmienda orgánica, *Psidium guajava*.

INTRODUCTION

Guava (*Psidium guajava* L.) (Myrtaceae) is a robust fruit-bearing tree originating from Central America that is cultivated in tropical and subtropical regions worldwide (Gonzaga Neto *et al*., 2001). Brazil ranks first in red guava production, with an annual output of over 300,000 tons of fruit, and a total cultivated area of about 15,000 hectares (ha) (Anonymous, 2010). In Brazil, the guava market is worth around US$ 38 million/year, which sustains thousands of small growers, family-operated facilities for pulp processing, and sales of pesticides, machinery and fertilizers.

Guava decline is a disease complex caused by the synergistic interaction between *Meloidogyne enterolobii* Yang and Eisenback, 1983 and *Fusarium solani* (Mart.) Sacc. (Gomes *et al*., 2011). In this disease complex, *F. solani*-immune guava trees suffer extensive root decay caused by this fungus following parasitism by *M. enterolobii*. Assays performed with root samples from different regions in Brazil confirmed that guava decline is the disease responsible for the decimation of about 5,000 ha of guava orchards nationwide, causing direct damage estimated of more than 70 million US$ (Pereira *et al*., 2009; Gomes *et al*., 2012).

Since parasitism by *M. enterolobii* is the predisposing factor for guava decline, the search for control or management alternatives of this disease focused on the nematode. Unsuccessful attempts involved fallowing, use of nematicides, and biological control using fungi, bacteria and entomopathogenic nematodes (Casassa *et al*., 1996; Gueye *et al*., 1997; Duponnois *et al*., 1998; Rodriguez *et al*., 2003; Carneiro *et al*., 2004; Souza *et al*., 2007; Molina *et al*., 2007; Almeida *et al*., 2011). Procedures for screening of *Psidium* spp. genotypes for resistance to *M. enterolobii* have been proposed (Burla *et al*., 2010; Miranda *et al*., 2010), and hundreds of accessions have been tested (Maranhão *et al*., 2001; 2003; Carneiro *et al*., 2007; Rodriguez *et al*., 2007; Almeida *et al*., 2009; Miranda, 2012). However, no resistant guava or rootstock cultivar is likely to be available to growers in the near future.

Several kinds of organic soil amendments have been used successfully to manage plant-parasitic nematodes, such as green covers, chitin-rich residues, municipal compost, waste sludge, and animal by-products (Stirling, 1991; Ferraz *et al*., 2010). Nonetheless, the efficacy of these amendments must be assessed for the pathosystem that one aims to manage. Other aspects to be considered are the availability and the cost of the amendment, the dosage and the application regime, and the profitability of the crop. For instance, Gomes *et al.* (2010) achieved promising results in guava decline-affected orchards upon soil amendment with poultry compost, combined with proper soil and foliar fertilization. Nonetheless, this management strategy was not adopted by local growers due to the relative difficulty in obtaining the compost.

Meat and bone meal (MBM) is a product of the rendering industry. It has been widely used as a source of protein and minerals in diets of production animals (Hendriks *et al.*, 2002), and its high content of organic matter, nitrogen, phosphorus and calcium has incited its assessment as fertilizer (e.g., Jeng *et al*., 2006). MBM has also been assessed as organic soil amendment to manage verticillium wilt, common scab and root lesion nematodes in potato fields, with modest results (Lazarovits *et al*., 1999; 2001). In greenhouse studies, Almeida *et al*. (2012) assessed different amendments against *M. enterolobii*, obtaining promising results for MBM at 3% v/v. For a preliminary field trial, this dosage was converted to 25 Kg/tree and tested in three application regimes (monthly, bimonthly or quarterly). All three regimes significantly ($P < 0.05$) reduced soil density of *M. enterolobii* second-stage juveniles (J_2), but there was no effect on plant productivity.

This article reports the results of a 2-year effort to further evaluate the efficacy of MBM to manage guava decline-affected orchards. To identify the best cost- and labor-effective scheme, different dosages and application regimes of MBM were assessed in one orchard. Furthermore, the most promising scheme (25 Kg/tree, quarterly) was assessed in three different orchards, which were distinct in age, levels of disease severity and agronomic input. A suite of variables was assessed, related to *M. enterolobii* and other soil plant-parasitic and free-living nematodes, *Fusarium* sp. density in soil and guava roots, release of ammonia in the soil upon MBM decomposition, and productivity in two harvests.

MATERIAL AND METHODS

Effect of Different Dosages and Application Regimes of MBM on Guava Decline

The experiment was established in a 10-year-old commercial guava 'Paluma' orchard, planted with 6 x 6 m spacing, in São João da Barra, State of Rio de Janeiro, Brazil (lat. 21°41'22"S; long. 41°3'20"W). The orchard was managed with annual prunings and irrigated with microsprinklers as needed. Fertilization was application of 60 Kg of cow manure/tree, under the canopy, twice a year, combined with hand-spread fertilization with nitrogen (N), phosphorus (P) and potassium (K) using the N-P-K formulation 20-5-20, at 300 g/tree, every three weeks. The incidence of rust, caused by *Puccinia psidii* Winter, and thrips was kept low with Folicur 200EC® and Folisuper 600 BR®, respectively, which are registered for this crop. Previous systematic samplings indicated an average density of 60 *M. enterolobii* J_2/100 cm^3 of soil, and a moderate severity of guava decline, with some trees showing chlorosis, scorched edges, wilting and falling of leaves, and root rot.

Three dosage/regime applications of MBM were tested: i) quarterly applications of 12.5 Kg/tree, ii) quarterly applications of 25 Kg/tree, and iii) semiannual applications of 50 Kg/tree. Untreated trees served as control. Concerns that ammonia released from MBM decomposition could drift laterally – despite the flat terrain - led to establishment of a second control plot in a nearby area in the same orchard. The four treatments were arranged in randomized blocks. Seventy-two trees were subdivided into six blocks of 12 trees each. Within each block, each treatment was represented by three plants, with only the middle one being assigned to data collection. Two planting rows were maintained as buffer between blocks. For MBM application, the organic debris below the canopy was removed manually with a rake. The MBM was hand-spread uniformly under the canopy, and superficially incorporated into the soil (0-5 cm) with the rake. The plant debris was returned and the trees were irrigated.

Routine chemical analysis of MBM indicated an average composition of 93.7% of dry matter, 41.5% of crude protein, 12% of crude fat, 38% of ash, 60 g/Kg of N, and 274 g/Kg of carbon.

Every 3 months, just before MBM applications, nematode density was evaluated under the canopy of each of the 30 trees assigned for data collection. Two soil samples (~ 500 cm^3) were collected on both sides of the canopy with an auger, at 0-20 cm depth, and homogenized. In the laboratory, roots and rootlets of each composite sample were separated and weighed. The number of *M. enterolobii*-induced galls were counted under magnifying lens and expressed as number of root galls/g of root and number of root galls/sample. One hundred cm^3 of soil was processed for nematode extraction following methods of Jenkins (1964). From the resulting nematode suspension, three aliquots of 0.25 ml were observed under the optical microscope using a Peters slide for nematode counting. The following variables were assessed: density of mycophagous, bacterivorous and predaceous nematodes, based on buccal cavity and esophagus morphology, *M. enterolobii* J_2, *Helicotylenchus dihysteroides* Siddiqi, 1972, and all plant-parasitic nematodes. In addition to high populations of *M. enterolobii* and *H. dihysteroides*, the orchard soil harbored also low populations of *Criconema* sp., *Mesocriconema* sp., *Pratylenchus* sp. and *Hemicycliophora* sp.

Soil density of colony-forming units (CFUs) of *Fusarium* sp. (at 0-20 cm depth) and soil concentration of ammonia (at 0-5 and 5-15 cm depths) were assessed in March 2012 for each of the 30 trees assigned for data collection, 15 days after MBM application. For fungal isolation, soil samples were collected as described before. A soil aliquot of 10 g was suspended in 100 ml of sterile water. Before sedimentation of the soil particles, an aliquot of 1 ml was pipetted out of the suspension and serially diluted from 10^{-4} through 10^{-6}. For each dilution, an aliquot of 0.1 ml was transferred to Petri plates containing the semi-selective medium (Martin, 1950), supplemented with 60 mg/ml of penicillin and 70 mg/ml of Rose Bengal. The Petri plates were incubated upside down for 3-7 days, at 28°C with a 12/12 h photoperiod. The resulting CFUs were counted under magnifying lens.

To assess the concentration of ammonia, soil samples (~50 cm^3) were collected with an auger as described previously. Immediately after sampling, the subsamples were homogenized and an aliquot of 10 g was placed in 50 ml plastic falcon tubes and kept cold in ice chest until processing. In the laboratory, 50 ml of deionized water was added to each tube and agitated. Ten ml of the supernatant was pipetted out and centrifuged at 1509 g for 20 min. After centrifugation, 2 ml of the supernatant was transferred to another glass tube, to which 6 ml of deionized water, 1 ml of disodium tartrate, and 1 ml of Nesler solution were added. After 10 min, the concentration of ammonia was determined through the spectrophometric method, with the aid of a spectrophotometer UV/VIS SPEKOL®, and a standard ammonium chloride solution (Van Standen and Taljaard, 1997). The concentration of ammonia was expressed in mg/Kg of soil.

For each of the 30 trees used for data collection, average productivity was assessed in two harvests (2009/2010 and 2010/2011). Guava fruits were hand-picked daily and productivity was expressed as kilograms of fruits/tree.

For all variables, data were transformed [log (x+1)] prior to analysis using ANOVA, and the treatment means were compared through Tukey test at 5%, using the software SAEG® (Ribeiro Júnior, 2001).

Effect of MBM on Orchards with Different Ages and Levels of Disease Severity and Agronomic Input

The experiment was established in three commercial 'Paluma' orchards located in two different properties in São João da Barra (lat. 21°39'21"S, long. 41°02'07"W; lat. 21°39'18"S, long. 41°02'02"W; lat 21°41'25"S, long 4103'23"W). Orchards 1, 2 and 3 were 10, 5 and 7 years old, respectively, and they were planted with 6 x 6 m spacing. The orchards were pruned, fertilized with N-P-K, and protected against rust and thrips as described previously.

Previous samplings indicated an average soil density of 57, 97 and 72 M. enterolobii J_2/100 cm^3 of soil in orchards 1, 2 and 3 respectively, and the trees had abundant root galls. At the beginning of the experiment, in orchard 1 the trees had little root rot and no symptoms in the shoot. In orchard 2, a few trees had leaf chlorosis and scorched edges. In orchard 3, several trees had abundant root rot, chlorosis, scorched edges, wilting and falling of leaves.

Local growers tend to reduce agronomical inputs when shoot symptoms are detected. In line with prevalent practices, orchard 1 was irrigated with sprinklers as needed, and fertilized with cow manure at 60 Kg/tree, under the canopy, twice a year. Orchard 2 was irrigated with microsprinklers daily for 2 hours, and fertilized with cow manure at 40 Kg/tree, twice a year. Orchard 3 received no organic fertilization, and was irrigated lightly with a hose, every other day.

In all orchards, two treatments were assessed: i) treatment with MBM at 25 Kg/tree quarterly and ii) no application of MBM (control). These treatments were arranged in randomized blocks. In each orchard, 36 trees were subdivided into six blocks of six trees each. Within each block, each treatment was represented by three trees, with only the middle one being assigned to data collection. Two planting rows were maintained as buffer between blocks. In all orchards, MBM application was performed as described previously.

For the variables assessed - soil and root density of *M. enterolobii*, soil density of nematode trophic groups, and productivity – data were collected and analyzed as described previously.

RESULTS

Table 1 shows the soil density of *H. dihysteroides* and four nematode trophic groups in relation to different dosages and application regimes of MBM, and the soil concentration of ammonia at two different soil depths. There was a reduction ($P < 0.05$) in the soil density of all plant-parasitic nematodes upon the quarterly application of 25 Kg of MBM/tree and the semiannual application of 50 Kg of MBM/tree, although there was no effect of MBM on *H. dihysteroides* in particular. The soil concentration of ammonia was low in all dosages and application regimes of MBM, with the exception of 50 Kg semiannualy at 0-5 cm depth.

Table 2 shows the variables related to guava decline proper, i.e., the density of *M. enterolobii* and *F. solani*, and the average productivity/tree in two harvests. For *M. enterolobii*, only the dosage of 25 Kg of MBM/tree reduced ($P < 0.05$) the soil density of J_2, although all dosages of MBM showed a tendency to reduce the J_2 density. No significant difference was observed among the treatments for the other variables.

For the experiment that evaluated the quarterly application of 25 Kg/tree of MBM in three distinct orchards, Table 3 shows the results of a factorial analysis highlighting the effect of MBM on *M. enterolobii*, and the average productivity/tree in two harvests. In each of three orchards, the quarterly application of 25 Kg/tree of MBM had no effect on nematode variables and productivity, in comparison with the untreated controls (results not shown). For all three orchards combined, the factorial analysis indicated no differences between MBM-treated and non-treated trees except for the variable density of *M. enterolobii* J_2/100 cm^3 of soil. For the same experiment, Table 4 shows the results of the factorial analysis highlighting the comparative productivity of the three orchards.

DISCUSSION

The unsatisfactory results observed in the present work for management of guava decline likely stem from the relatively low concentration of ammonia observed in the soil upon MBM decomposition, with the exception of the 50 Kg/tree dosage at 0-5 cm depth. Indeed, ammonia was reported to have a nematicidal effect when present in soil at 300 mg/Kg of soil (Eno *et al*., 1955; Rodriguez-Kabana *et al*., 1981; 1982). As reported by these and many other authors, in the present work the input of organic matter resulted in an increase ($P < 0.05$) in the soil density of bacterivorous nematodes. This likely occurred following an increase in the soil density of bacterial CFUs upon application of MBM, as observed by Almeida *et al*. (2012).

Table 1. Nematode population density (100 cm^3 of soil) and concentration of ammonia (in mg/Kg of soil) upon soil application of meat and bone meal (MBM) at different doses and application regimes, in a commercial guava orchard affected by guava decline, in São João da Barra, Brazil.

Treatments	*Helicotylenchus dihysteroides*	Plant-parasitic nematodes	Bacterivorous nematodes	Mycophagous nematodes	Predaceous nematodes	Ammonia concentration[x] 0-5 cm depth	Ammonia concentration[x] 5-15 cm depth
Untreated control[y]	28.3 a[w]	98.1 a	147.5 b	20.0 a	28.3 a	2.7 c	2.2 b
Untreated control[z]	28.3 a	92.2 a	241.7 b	15.5 a	28.3 a	2.9 c	1.6 b
12.5 Kg/tree, quarterly	23.1 a	46.7 a	461.1 a	26.7 a	23.1 a	1.9 c	0.2 c
25 Kg/tree, quarterly	23.3 a	37.2 b	466.7 a	21.1 a	23.3 a	38.7 b	3.2 b
50 Kg/tree semiannualy	26.7 a	47.2 b	508.9 a	20.0 a	26.7 a	221.2 a	17.8 a
CV (%)	68.8	45.4	15.7	77.1	56.8	16.8	34.9

[x]Determined once in March 2012. Values are average of six guava trees/treatment. Soil samples were taken 15 days after MBM application.
[y]Guava decline-affected trees located within the experimental plot.
[w]Values are average of six guava trees/treatment, sampled quarterly for 24 months, just before each MBM application. Values followed by same letters in the column are not different at $P < 0.05$
[z]Guava decline-affected trees located outside the experimental plot, in the same orchard.

Table 2. Variables related to *M. enterolobii*, incidence of *Fusarium* sp., and productivity upon soil application of meat and bone meal (MBM) at different doses and application regimes, in a commercial guava orchard affected by guava decline, in São João da Barra, Brazil.

Treatments	J_2/100 cm^3 of soil	Root galls/ sampling	Root galls/g of root	CFUs[x] of *Fusarium* sp./g of soil	Root fragments positive for *Fusarium* sp. (%)	Productivity (Kg of fruit/tree)
Untreated control[y]	56.4 ab[w]	102.4 a	30.9 a	397.8 a	31.1 a	126.4 a
Untreated control[z]	71.7 a	105.5 a	38.9 a	818.0 a	31.1 a	Not assessed
12.5 Kg/tree, quarterly	25.5 bc	179.7 a	54.0 a	713.6 a	51.1 a	109.8 a
25 Kg/tree, quarterly	17.2 c	73.2 a	44.5 a	11,839 a	35.5 a	159.0 a
50 Kg/tree semiannualy	35.5 bc	79.6 a	32.6 a	5,764 a	33.3 a	119.2 a
CV (%)	60.7	33.7	32.3	140.9	67.8	36.4

[x]Colony forming units.
[y]Guava decline-affected trees located within the experimental plot.
[w]Values are average of six guava trees/treatment, sampled quarterly for 24 months, just before each MBM application. Productivity is the average of two harvests. Values followed by same letters in the column are not different at $P< 0.05$
[z]Guava decline-affected trees located outside the experimental plot, in the same orchard.

Table 3. Factorial analysis of variables related to *M. enterolobii* and productivity in three commercial guava orchards affected by guava decline, upon quarterly soil application of 25 Kg/tree of meat and bone meal, in São João da Barra, Brazil.

Treatments	Density of J^2/100 cm^3 of soil	Density of root galls/sampling	Density of root galls/g of root	Productivity (Kg of fruit/tree)
Treated	41.9 b[z]	47.9 a	37.2 a	84.8 a
Untreated (control)	77.1 a	54.9 a	45.1 a	65.5 a
CV (%)	61.5	36.8	38.5	54.6

[z]Values are average of 18 guava trees/treatment, sampled quarterly for 24 months, just before each MBM application. Productivity is the average of two harvests. Values followed by same letters in the column are not different at $P< 0.05$.

Table 4. Factorial analysis of productivity in guava decline-affected orchards in São João da Barra, Brazil. Orchards 1-3 presented different ages and levels of disease severity and agronomic input upon start of the experiment.

Treatments	Productivity (Kg of fruit/tree)	Productivity increase (in %) in relation to orchard 3
Orchard 1	153.5 a[z]	+634.3
Orchard 2	47.5 b	+127.5
Orchard 3	20.9 b	-
CV (%)	54.6	-

[z]Values are average of 12 guava trees/treatment, in two harvests. Values followed by same letters in the column are not different at $P< 0.05$.

Contrary to the observation reported by Gomes *at al.* (2010), the variables involving density of *M. enterolobii*-induced root galls did not relate to J_2 density in the soil, i.e., in all treatments the guava trees were heavily parasitized with as many as 54 galls/g of root. Consequently, the conspicuous presence of *Fusarium* sp. in the roots and soil led to an increasing severity of guava decline during the experiment, with several trees advancing the sequence of symptoms described before. Therefore, the low and statistically similar productivity of all treatments comes as no surprise. The use of MBM in three different orchards showed that age and the level of disease severity at the beginning of the experiment, and the corresponding level of agronomic input during the 24-month period of the experiment, were the key factors determining the differences ($P < 0.05$) observed in the orchards' productivity, which were in excess of 600%.

In conclusion, soil application of as much as 50 Kg/tree of MBM resulted in only marginal reduction of *M. enterolobii* population. This could stem from the relatively low concentration of ammonia in the soil upon MBM decomposition. Most likely ammonia was lost to the atmosphere and/or lixiviated by irrigation water because of the sandy nature of the soil (98% quartz sand). MBM holds potential, nonetheless, for non-complex diseases involving plant-parasitic nematodes, either in perennial or annual horticultural crops. In the São João da Barra area, MBM is presently sold to growers at US$ 0.15/Kg, which is a reasonable cost considering its nematicidal potential and high content of organic matter, nitrogen, phosphorus and calcium.

Most importantly, the present work shows how intractable plant-parasitic nematodes can be when involved in a disease complex, in which case management through the use of soil organic amendments may not be advisable. Indeed, any effective control or management strategy would need to significantly reduce parasitism - viz, root galls and nematode feeding females in the case of *Meloidogyne* spp. - to minimize the alterations in plant physiology that allow the synergistic interaction to occur between the nematode and the fungus or bacterium. For instance, in guava decline the parasitic capability of *F. solani* towards guava roots seems to be mediated by chemical alteration(s) in exudate released from gall-laden roots (Gomes, 2011).

Since no nematicide is registered for use in guava orchards to promote significant decrease in *M. enterolobii*-parasitism, nematode resistance seems the only feasible strategy to control guava decline (Miranda *et al*., 2010). So far, a major obstacle towards this goal has been the non-existence of *M. enterolobii*-resistant guavas, and the grafting incompatibility between guava cultivars and a few *M. enterolobii*-resistant *Psidium* sp. genotypes, which hampers the release of resistant rootstocks.

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