Improved nutrient uptake in three Crotalaria species inoculated with multifunctional microorganisms

Melhoria da absorção de nutrientes em três espécies de Crotalaria inoculadas com microorganismos multifuncionais

Anna C. Lanna2*, Mariana A. Silva3, Alécio S. Moreira4, Adriano S. Nascente2 & Marta C. C. de Fillipi2

ABSTRACT: Cover crops are essential in recovering soil productivity. Crotalaria is one of the most efficient legume species in terms of biomass production and nitrogen fixation. This study aimed to assess the effect of multifunctional microorganisms on the agronomic performance of Crotalaria juncea, C. spectabilis and C. ochroleuca. The experiment was conducted under greenhouse conditions, in a completely randomized design, with four replicates. Treatments consisted of six rhizobacterial isolates (BRM 32109 and BRM 32110 (Bacillus spp.), BRM 32111 and BRM 32112 (Pseudomonas spp.), BRM 32113 (Burkholderia spp.), BRM 32114 (Serratia spp.)), and one fungal isolate (Trichoderma spp. (T-26)), in addition to a control treatment (no microorganism). The main effect of multifunctional microorganisms on the three Crotalaria species was macro and micronutrient concentration increased. Sulfur and zinc concentrations increased in C. juncea roots, calcium and sulfur in C. spectabilis shoots, and C. ochroleuca exhibited higher concentrations of phosphorus and copper in shoots and zinc and copper in roots. In summary, improved nutritional status in Crotalaria directly affects nutrient availability for the subsequent crop.

Key words: plant growth promotion, biomass, nutrient, gas exchange

HIGHLIGHTS:
Multifunctional microorganisms promote the nutrient enrichment in Crotalaria plants.
Cover crop residues are vital in managing soil fertility.
Nutritionally improved cover crops increase soil nutrient levels for the subsequent crop.

RESUMO: Plantas de cobertura são essenciais na recuperação da produtividade do solo. Crotalaria é uma das mais eficientes espécies de leguminosas em termo de produção de biomassa e fixação de nitrogênio. Objetivou-se neste estudo avaliar o efeito de microorganismos multifuncionais no desempenho agronômico de Crotalaria juncea, C. spectabilis e C. ochroleuca. O experimento foi conduzido em casa de vegetação, em delineamento inteiramente casualizado, com quatro repetições. Os tratamentos consistiram em seis isolados de rizobactérias (BRM 32109 e BRM 32110 (Bacillus spp.), BRM 32111 e BRM 32112 (Pseudomonas spp.), BRM 32113 (Burkholderia spp.), BRM 32114 (Serratia spp.)), e um isolado fúngico (Trichoderma spp. (T-26)), além do tratamento controle (sem microrganismo). O principal efeito dos microorganismos multifuncionais sobre as três espécies de Crotalaria foi o aumento da concentração de macro e micronutrientes. Enxofre e zinco aumentaram na raiz de plantas de C. juncea; cálcio e enxofre na parte aérea de plantas de C. spectabilis; e plantas de C. ochroleuca apresentaram maior concentração de fósforo e cobre na parte aérea e de zinco e cobre na raiz. Em resumo, o melhor status nutricional em plantas de Crotalaria afeta diretamente a disponibilidade de nutrientes para a cultura subsequente.

Palavras-chave: promoção do crescimento vegetal, biomassa, nutrientes, trocas gasosas

1 Research developed at Embrapa Arroz e Feijão, Santo Antônio de Goiás, GO, Brazil
2 Empresa Brasileira de Pesquisa Agropecuária/Embrapa Arroz e Feijão, Santo Antônio de Goiás, GO, Brazil
3 Universidade Federal de Goiás/Faculdade de Agronomia, Goiânia, GO, Brazil
4 Empresa Brasileira de Pesquisa Agropecuária/Embrapa Mandioca e Fruticultura, Araraquara, SP, Brazil
**Introduction**

*Crotalaria* spp. is a legume used as a cover crop in a no-till systems in order to protect the soil against erosion and increase organic matter and nutrient accumulation (Aita & Giacomini, 2006; Pereira et al., 2016). *Crotalaria* species are also used to reduce the incidence of phytonematodes in the soil (Pacheco et al., 2015) and break up compacted layers due to their deeper and more branched root system (Bonfim-Silva et al., 2012), in addition to fixing nitrogen (200 to 300 kg ha⁻¹) through a symbiotic relationship with bacteria of the genus *Rhizobium* (Dourado et al., 2001). Rotating cash crops with leguminous cover crops in the off season (March to September) is widespread in Brazilian Cerrado agricultural systems (Boer et al., 2007).

The use of multifunctional microorganisms in symbiosis with the cover crop is essential for sustainable intensification of agricultural systems in the Brazilian Cerrado region. These microorganisms usually inhabit the rhizosphere of plants (Graças et al., 2015) and improve crop systems resilience by promoting plant growth through direct and indirect mechanisms, in addition to increasing plant protection against pathogens and insects (Ahemad & Kilbret, 2014).

Previous studies conducted at Embrapa Rice and Beans research center demonstrated the efficiency of microorganisms, rhizobacteria and fungi in increasing biomass production and disease resistance in upland rice (Filippi et al., 2011; Silva et al., 2012; França et al., 2015; Nascente et al., 2017). In a more recent study, micronutrients, fulvic acid and *Ascosphyllum* promoted total dry matter accumulation and a larger number of pods per plant in the common bean, an important legume (*C. juncea*, *C. spectabilis* and *C. ochroleuca*) in individual experiments. Treatments consisted of six rhizobia isolates (Brasil spp. (BRM 32109 and BRM 32110), *Pseudomonas* spp. (BRM32111), *Pseudomonas fluorescens* (BRM 32112), *Burkholderia pyrocinia* (BRM 32113), *Serratia* spp. (BRM32114)) (Table 1), one fungal isolate (*Trichoderma* spp. (T-26)) (biochemical characterization and taxonomic classification underway) and a control. The microorganisms were selected from upland rice fields and are currently stored and preserved in the Multifunction Microorganisms and Fungi Collection of Embrapa Rice and Beans. The control treatment consisted solely of water, with no microorganisms applied. The microorganisms were applied at three moments: (1) seed microbiolization, (2) soil drenched with microbial suspension 10 days after sowing (DAS) and (3) plants sprayed with microbial suspension at 21 DAS.

The rhizocterial isolates were grown on solid medium 523 (Kado & Heskett, 1970), at 28 °C, for 24 hours. The concentration was set to A540 = 0.5 (10⁴ CFU, colony-forming units), in a spectrophotometer. *Trichoderma* spp. was grown in a Petri dish containing potato dextrose agar (PDA) for 5 days and suspensions were prepared and bioformulated as described by Silva et al. (2012). The concentration of the biological suspension was 10⁶ conidia ml⁻¹.

For microbiolization, *Crotalaria* seeds were immersed in each microorganism suspensions, and control seeds in water, for 24 hours under constant agitation at 25 °C.

For soil drenching, 100 mL of the suspension of each treatment and water (control treatment) were applied to the soil at 10 DAS.

For plant spraying, 30 mL of the suspension of each treatment and water (control treatment) were sprayed onto leaves at a constant pressure, using a CO₂ pressurized manual backpack sprayer equipped with a hollow-cone spray nozzle (TX-VS2), was performed at 21 DAS.

Gas exchange was measured by using a portable gas exchange analyzer in the infrared region (LCpro+, ADC BioScientific, Hoddesdon, England). Photosynthetic rate (A, μmol CO₂ m⁻² s⁻¹), transpiration rate (E, mmol H₂O m⁻² s⁻¹), stomatal conductance (gs, mol H₂O m⁻² s⁻¹), internal CO₂

**Table 1.** Isolate code, origin, biochemical characteristics and taxonomic classification of six rhizobacterial isolates

| Isolate code | Origin | Color   | Biochemical characteristics | Taxonomic class          |
|--------------|--------|---------|----------------------------|--------------------------|
| BRM 32109    | GO/Brazil | White  | +                          | *Bacillus* spp.          |
| BRM 32110    | PA/Brazil | White  | +                          | *Bacillus* spp.          |
| BRM 32112    | GO/Brazil | Yellow | +                          | *Pseudomonas* spp.       |
| BRM 32113    | PA/Brazil | Pink   | +                          | *Burkholeria* spp.       |
| BRM 32114    | PA/Brazil | Pink   | +                          | *Serratia* spp.          |

¹Isolate code of the rhizobacterial and fungal isolates in the Multifunctional Microorganism and Fungi Collection of Embrapa Rice and Beans collection; ²Geographical origin of each isolate; ³Colony color; ⁴Biochemical characterization and taxonomics classification of each isolate; ⁵Indol acetic acid producer; ⁶Cellulase producer; ⁷Phosphatase producer; ⁸Siderophore producer; ⁹Exopolysaccharides producer

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concentration (Ci, μmol mol⁻¹) and leaf temperature (Tleaf, °C) were obtained. Readings were taken between 08:00 and 10:00 a.m., at 65 days after emergence (DAE). Samples were collected from the middle third of the youngest fully expanded leaves on the main stem. The equipment was set to use concentrations of 370-400 mol mol⁻¹ CO₂ in the air, which is the reference condition used in the IRGA photosynthesis chamber. The photon flux density photosynthetic active (PPFD) used was 1200 μmol [quanta] m⁻² s⁻¹. The minimum equilibration time set for performing the reading was 2 min.

Shoot and root dry weight for each plot (one plant per pot) were determined in the full flowering stage. The shoots and roots were washed in water, dried in a forced-air circulation oven at 65 °C for 72 hours and, then, weighed. After weighing, the dried shoot and root samples were ground and P, K, Ca, Mg, S, Cu, Fe, Mn, Zn and Mo concentrations determined as described by Donagema et al. (2011). Fe was only quantified in the shoots.

Most of the data showed normal distribution, with exception of shoot Mn (C. juncea), Fe and Mo (C. spectabilis) and Fe (C. ochroleuca) concentration and root Cu, Mn and Zn (C. juncea), K, Ca, Mg, S and Mn (C. spectabilis) and P, Ca, Mn, Zn and Mo (C. ochroleuca) concentration. These data were log-transformed (Log10 Y) for statistical analysis. The transformed and non-transformed data were submitted to analysis of variance (ANOVA). Tukey’s test was performed for each Crotalaria species, at p ≤ 0.05. Sisvar software 5.1 was used for statistical analyses (Ferreira, 2011).

### Results and Discussion

No significant gas exchange effects caused by microorganisms were observed in Crotalaria plants under the experimental conditions. The respective values obtained for A, E, gs (number and activity of stomata) and Ci ranged from 10.34 to 18.99 μmol CO₂ m⁻² s⁻¹, 1.61 to 2.14 mmol H₂O m⁻² s⁻¹, 0.11 to 0.25 mol H₂O m⁻² s⁻¹ and 156 to 242 μmol mol⁻¹ for C. juncea, 15.74 to 21.46 μmol CO₂ m⁻² s⁻¹, 2.82 to 4.58 mmol H₂O m⁻² s⁻¹, 0.19 to 0.26 mol H₂O m⁻² s⁻¹ and 185 to 235 μmol mol⁻¹ for C. spectabilis, and 13.07 to 25.69 μmol CO₂ m⁻² s⁻¹, 2.67 to 5.27 mmol H₂O m⁻² s⁻¹, 0.15 to 0.31 mol H₂O m⁻² s⁻¹ and 179 to 21 μmol mol⁻¹ for C. ochroleuca. Leaf temperature ranged from 28.0 to 35.3 °C during assessment of the three Crotalaria species. According to Lang et al. (2015), it is essential to determine the influence of multifunctional microorganisms on plant physiology. This can be achieved by monitoring plant health and increased photosynthate, biomass and grain production. The effect of multifunctional microorganisms on plant physiology is particularly evident under stressed conditions (Ahemad & Kilbret, 2014).

In addition, there was no significant effect on shoot and root phytomass accumulation in C. juncea, C. spectabilis and C. ochroleuca compared to their respective controls (Tables 2 and 3). However, C. spectabilis and C. ochroleuca differed between microorganism treatments. C. spectabilis treated with BRM 32113 exhibited higher SDMB (28.79 g) than that of

### Table 2. Shoot dry matter biomass (SDMB) and shoot macro/micronutrients concentration of C. juncea, C. spectabilis and C. ochroleuca, grown in soil containing multifunctional microorganisms

| Treatment | SDMB (g plant⁻¹) | Macronutrients | Micronutrients |
|-----------|------------------|----------------|---------------|
|           |                  | P (g kg⁻¹)     | K (g kg⁻¹)    | Ca (g kg⁻¹)  | Mg (g kg⁻¹)  | S (g kg⁻¹) | Cu (mg kg⁻¹) | Fe (mg kg⁻¹) | Mn (mg kg⁻¹) | Mo (μg kg⁻¹) |
| C. juncea |                  |                |               |              |              |            |              |              |              |              |
| 32109     | 25.73 ab         | 2.34 ab        | 14.09 ab      | 13.77 ab     | 2.18 ab      | 3.33 a      | 5.87 a       | 600.03 c     | 80.30 a       | 65.72 a       |
| 32110     | 14.00 c          | 2.77 a         | 13.35 ab      | 13.94 ab     | 2.68 ab      | 3.46 a      | 6.76 a       | 221.96 c     | 86.05 a       | 62.16 a       |
| 32111     | 17.88 bc         | 2.33 ab        | 11.84 ab      | 14.31 ab     | 2.62 ab      | 3.60 a      | 6.56 a       | 230.95 c     | 96.43 a       | 62.62 a       |
| 32112     | 22.12 abc        | 2.45 ab        | 11.67 ab      | 13.94 ab     | 2.48 ab      | 3.42 a      | 5.81 a       | 143.96 d     | 74.53 a       | 58.89 a       |
| 32113     | 28.70 a          | 2.72 a         | 13.17 ab      | 14.56 ab     | 2.30 ab      | 3.30 a      | 6.88 a       | 426.63 c     | 76.40 a       | 67.53 a       |
| 32114     | 17.21 bc         | 2.33 ab        | 15.00 ab      | 13.77 ab     | 2.47 ab      | 3.30 a      | 6.12 a       | 280.09 c     | 116.09 a      | 67.79 a       |
| T-6       | 18.85 bc         | 2.08 b         | 12.32 ab      | 11.35 ab     | 2.35 ab      | 3.08 a      | 5.49 a       | 298.57 c     | 83.29 a       | 56.33 a       |
| Control   | 24.69 ab         | 2.15 ab        | 11.42 ab      | 11.03 b      | 2.23 ab      | 2.73 b      | 6.60 a       | 204.56 c     | 79.79 a       | 57.31 a       |
| CV (%)    | 20.4             | 11.5           | 12.5          | 10.6         | 10.7         | 9.7         | 11.9         | 11.7         | 29.1          | 19.1          |
| C. ochroleuca |              |                |               |              |              |            |              |              |              |              |
| 32109     | 24.91 cd         | 2.08 abc       | 14.57 ab      | 6.68 ab      | 3.03 ab      | 2.91 ab     | 5.59 ab      | 411.56 c     | 77.76 a       | 65.34 a       |
| 32110     | 10.80 e          | 1.72 bc        | 13.23 ab      | 5.76 ab      | 2.30 ab      | 2.96 ab     | 3.17 b       | 129.56 c     | 78.10 a       | 52.60 a       |
| 32111     | 40.16 a          | 1.54 c         | 16.16 ab      | 5.39 ab      | 2.94 ab      | 3.22 ab     | 4.27 ab      | 298.46 c     | 76.76 a       | 55.71 a       |
| 32112     | 28.28 bcd        | 1.83 bc        | 14.48 a       | 6.89 ab      | 3.00 ab      | 3.06 ab     | 4.07 ab      | 267.76 d     | 61.81 a       | 49.05 a       |
| 32113     | 22.51 cd         | 2.64 a         | 14.98 ab      | 6.74 ab      | 3.07 ab      | 3.78 a      | 6.20 a       | 222.23 c     | 75.90 a       | 58.61 a       |
| 32114     | 17.73 de         | 1.93 abc       | 15.12 ab      | 8.62 ab      | 3.14 ab      | 3.63 a      | 4.48 ab      | 311.98 c     | 64.42 a       | 61.65 a       |
| T-6       | 32.32 abc        | 2.43 ab        | 15.50 ab      | 8.19 ab      | 3.36 ab      | 3.50 a      | 5.46 ab      | 199.31 c     | 81.71 a       | 77.58 a       |
| Control   | 37.41 ab         | 1.39 c         | 14.23 a       | 5.94 ab      | 2.87 ab      | 2.73 ab     | 3.25 b       | 113.48 b     | 55.06 a       | 67.71 a       |
| CV (%)    | 14.9             | 12.8           | 11.3          | 11.8         | 12.7         | 11.9        | 10.6         | 18.2         | 21.1          | 28.0          |

Different letters in columns indicate significant differences at p ≤ 0.05 by the Tukey test. Shoot data for Mn (C. juncea), Fe and Mo (C. spectabilis) and Fe (C. ochroleuca) were log-transformed (Log10 Y) for statistical analysis.
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Table 3. Root dry matter biomass (RDMB) and root macro/micronutrients concentration of *C. juncea*, *C. spectabilis* and *C. ochroleuca*, grown in soil containing multifunctional microorganisms

| Treatment | RDMB (g plant⁻¹) | Macronutrients (g kg⁻¹) | Micronutrients (µg kg⁻¹) | *C. juncea* | *C. spectabilis* | *C. ochroleuca* |
|-----------|------------------|-------------------------|--------------------------|-------------|-----------------|----------------|
| 32109     | 5.11 a           | 1.98 a                  | 18.10 a                  | 10.29 a     | 2.87 a          | 6.12 a         |
| 32110     | 7.29 a           | 2.09 a                  | 19.25 a                  | 11.41 a     | 2.64 a          | 5.82 b         |
| 32111     | 8.06 a           | 1.70 a                  | 12.49 a                  | 5.25 a      | 1.94 a          | 3.86 b         |
| 32112     | 5.18 a           | 2.01 a                  | 18.17 a                  | 8.28 a      | 2.79 a          | 5.82 b         |
| 32113     | 6.08 a           | 1.93 a                  | 15.21 a                  | 8.50 a      | 2.24 a          | 4.93 b         |
| 32114     | 7.04 a           | 1.87 a                  | 15.23 a                  | 5.46 a      | 2.16 a          | 4.22 b         |
| T-26      | 5.30 a           | 1.97 a                  | 17.03 a                  | 9.17 a      | 2.77 a          | 5.61 b         |
| Control   | 8.32 a           | 1.40 a                  | 11.20 a                  | 6.01 a      | 1.73 a          | 3.99 b         |
| CV (%)    | 22.0             | 20.3                    | 30.2                     | 32.9        | 23.6            | 25.8           |

Different letters in columns indicate significant differences at p ≤ 0.05 by the Tukey test; Root data for Cu, Mn and Zn (*C. juncea*), K, Ca, Mg, S and Mn (*C. spectabilis*) and Ca, Mn, Zn and Mo (*C. ochroleuca*) were log-transformed (Log10 Y) for statistical analysis.

Plants treated with BRM 32110 (14.00 g), BRM 32111 (17.88 g), BRM 32114 (17.21 g) and T-26 (18.85 g) (Table 2), whereas the highest SDMB value for *C. ochroleuca* was observed in the BRM 32111 treatment (40.16 g), with values of 24.91, 10.80, 28.28, 22.51 and 17.73 g for BRM 32109, BRM 32110, BRM 32112, BRM 32113 and BRM 32114, respectively. *C. ochroleuca* treated with BRM 32111 exhibited higher RDMB (26.66 g) than that obtained for the other treatments (Tables 2 and 3). Additionally, BRM 32110, BRM 32113 and BRM 32114 displayed reduced root biomass (5.70, 8.62 and 4.48, respectively), differing from the control treatment. Root and shoot biomass are important in cover crops because they represent better protection against soil erosion, nutrient cycling, breaking compacted soil layers and lower soil temperature when compared to conventional tillage (Nascente et al., 2013). With respect to shoot macro and micronutrient concentration, *C. spectabilis* plants treated with multifunctional microorganisms exhibited increased S concentration (23%) and Ca concentration (31%) in relation to the control (Table 2). *C. ochroleuca*, shoot concentration of P rose by 83% after inoculation with BRM 32113 and T-26 (*Trichoderma spp.*), and Cu concentration by 91% when compared to the control treatment. Shoot macro and micronutrient concentrations were similar between treatments for *C. juncea*.

According to Baldotto et al. (2010), fresh and dry matter of the root and shoot systems increased in pineapple plantlets inoculated with *Burkholderia*, resulting in 115, 112 and 69% higher N, P and K concentrations, respectively, than those obtained in controls. The genus *Burkholderia* includes phytopathogenic bacteria (Burkholder, 1950), endophytic diazotrophic bacteria (Perin et al., 2006) and symbiotic strains of the beta-rhizobia group that induce the formation of nitrogen-fixing root nodules in host plants (Rasolomampianina et al., 2005). The *Burkholderia* spp. used in the present study exhibited cellulase activity, in addition to producing IAA (indole-3-acetic acid) and siderophores (Table 1). Nevertheless, other studies have reported additional biochemical characteristics for *Burkholderia*, including phosphate solubilization (Ghosh et al., 2016), ACC deaminase activation (Onofre-Lemus et al., 2009) and biocontrol (Esmaeel et al., 2020).

Based on these results, microorganisms seem to stimulate greater nutrient availability in the soil solution, leading to accumulation of these nutrients in the shoots of *Crotalaria* plants (Pérez-Garcia et al., 2011; Zhang et al., 2011). Teodoro et al. (2011) reported that different herbaceous leguminous species, including *Crotalaria* spp., demonstrated potential for nutrients recycling and N input (approximately 19.94 kg ha⁻¹ of N) in crop production systems in the Brazilian Cerrado.

With regard to root macro and micronutrient concentration, S concentration rose by 98% and Zn by 135% in *C. juncea*. According to Baldotto et al. (2010), fresh and dry matter of the root and shoot systems increased in pineapple plantlets inoculated with *Burkholderia*, resulting in 115, 112 and 69% higher N, P and K concentrations, respectively, than those obtained in controls. The genus *Burkholderia* includes phytopathogenic bacteria (Burkholder, 1950), endophytic diazotrophic bacteria (Perin et al., 2006) and symbiotic strains of the beta-rhizobia group that induce the formation of nitrogen-fixing root nodules in host plants (Rasolomampianina et al., 2005). The *Burkholderia* spp. used in the present study exhibited cellulase activity, in addition to producing IAA (indole-3-acetic acid) and siderophores (Table 1). Nevertheless, other studies have reported additional biochemical characteristics for *Burkholderia*, including phosphate solubilization (Ghosh et al., 2016), ACC deaminase activation (Onofre-Lemus et al., 2009) and biocontrol (Esmaeel et al., 2020).

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inoculated with BRM 32109 and BRM 32110, respectively, when compared to the control treatment (Table 3). Concentrations of Cu and Zn rose by 126 and 136% in *C. ochroleuca* treated with BRM 32110 and BRM 32114, respectively, in relation to the control. In *C. spectabilis* plants, root macro and micronutrient concentrations were similar between treatments.

Soil with low fertility predominates in tropical areas, making soil fertility management essential in maintaining an economically and environmentally sustainable farming system. The use of green manures and crop residues exerts different conditioning effects on the soil; however, the main objectives of this practice in low-fertility tropical soils are to improve the cation exchange capacity (CEC) and provide nutrients such as Ca, Mg, K and trace elements. Additionally, the decomposition of residues and OM releases nutrients such as Ca, Mg, K and trace elements (Valadares et al., 2016).

Overall, the three *Crotalaria* species treated with multifunctional microorganisms, showed no significant differences in agronomic performance, except for increased shoot and root nutrient accumulation. This is essential to ensure greater nutrient availability for subsequent crops in soil that typically exhibits low fertility.

**Conclusions**

1. Multifunctional microorganisms, selected from upland rice fields, improved the nutritional status of *Crotalaria juncea*, *C. spectabilis* and *C. ochroleuca*.

2. In shoots, Ca concentration increased in *C. spectabilis* inoculated with BRM 32111 and BRM 32113, and those treated with multifunctional microorganisms generally showed higher S concentrations. *C. ochroleuca* treated with BRM 32111 and T-26 exhibited higher P concentration and higher Cu concentration when inoculated with BRM 32113.

3. In roots, S and Zn concentrations were higher in *C. juncea* treated with BRM 32109 and BRM 32110, respectively; while *C. ochroleuca* inoculated with BRM 32110 and BRM 32114 showed greater Cu and Zn concentrations, respectively.

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