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CROP PRODUCTION

Host suitability of weeds to *Meloidogyne ottersoni* and *Meloidogyne graminicola*

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ABSTRACT: We evaluated the host suitability of the weeds associated with rice crops regarding *Meloidogyne ottersoni* and *M. graminicola*. Both plant-parasitic nematodes can develop in *Oryza sativa*, but *Cyperus ferax* plants were resistant to *M. ottersoni*. Plants of *Cyperus iria*, *Cyperus difformis*, *Echinochloa crus-galli* and *Echinochloa colonum* were susceptible to *M. ottersoni*, but resistant to *M. graminicola*. Besides this, *Aeschynomene denticulata* and *Leersia hexandra* were immune to *M. graminicola* and susceptible (1st assessment) and resistant (2nd assessment) regarding *M. ottersoni*. The results shed light on the role of hosts of *M. ottersoni* and *M. graminicola*, demonstrating that weed management should be included in strategies to control root-knot nematode diseases.
Key words: management of diseases, rice, plant-parasitic nematodes.

Reação de plantas daninhas a *Meloidogyne ottersoni* e *Meloidogyne graminicola*

RESUMO: Objetivou-se avaliar a reação de plantas daninhas associadas à cultura de arroz em relação a *Meloidogyne ottersoni* e *M. graminicola*. Ambos fitonematoides podem se desenvolver em *Oryza sativa*, mas plantas de *Cyperus ferax* foram resistentes a *M. ottersoni*. Plantas de *Cyperus iria*, *Cyperus difformis*, *Echinochloa crus-galli* e *Echinochloa colonum* foram suscetíveis a *M. ottersoni*, mas resistentes a *M. graminicola*. Além disso, *Aeschynomene denticulata* e *Leersia hexandra* comportaram-se como imunes a *M. graminicola* e suscetíveis (1ª avaliação) e resistentes (2ª avaliação) em relação a *M. ottersoni*. Os resultados ampliam o conhecimento acerca de plantas hospedeiras de *M. ottersoni* e *M. graminicola*, demonstrando que controle de plantas daninhas deve ser incorporado nas estratégias de manejo de meloidoginoses.
Palavras-chave: manejo de doenças, arroz, fitonematoides.

INTRODUCTION

Rice (*Oryza sativa* L.) growing is hugely important to produce food, employment and income by millions of people around the world. Among the leading producing countries in 2022 were continental China (208.5 million tons), followed by India (196.2 million tons). Brazil was in eleventh place, with an output of 10.8 million tons (FAO, 2024) and Brazil's South region stands out in the production of irrigated rice, with the state of Rio Grande do Sul being the leading producer (7.29 million tons), followed by Santa Catarina (1.12 million tons) (IBGE, 2024).

Among the phytosanitary factors that limit the productivity of rice are attacks by pests and encroachment of weeds (SAVARY et al., 2012; AVILA et al., 2021). The losses caused by weeds to rice crops can be direct (competition) and indirect, due to the multiplication/maintenance of various pathogens (FERRAZ et al., 1983; AGOSTINETTO et al., 2008; SILVA et al., 2010; CONCENÇO et al., 2014). Globally, *M. graminicola* GOLDEN & BIRCHFIEL, 1965, is the species with the greatest potential to damage irrigated rice crops (DE WAELE & ELSEN, 2007). Besides rice, various plants present in fields between harvests can serve as hosts, such as *Echinochloa colonum* (L.) Link. (GOLDEN & BIRCHFIELD, 1965), *E. crus-galli* (L.) P. Beauv., 1812, *Eleusine indica* (L.) Gaerth., 1788, and *Cyperus difformis* L., 1756 (BAJAJ & DABUR, 2000; DABUR et al., 2004; NEGRETTI et al., 2014; KUMAR et al., 2019), as well as *Juncus microcephalus* Kunth, 1816 (BELLÉ et al., 2021).

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Various studies have been carried out to investigate weeds commonly found in rice fields as hosts of *M. graminicola* (RUSINQUE et al., 2021). In Brazil, the ability of weeds to host *M. graminicola* was initially reported by MONTEIRO & FERRAZ (1988), in *C. ferax* L.C. Rich., 1792, but it was only in the 1990s that this plant-parasitic nematode was reported in various species of native and cultivated plants in the state of Rio Grande do Sul (SPERANDIO & MONTEIRO, 1991; SPERANDIO & AMARAL, 1994). Several *Meloidogyne* species have been found in rice-growing areas of Rio Grande do Sul, Santa Catarina and Paraná, among them *M. graminicola* (SOARES et al., 2020), *M. ottersoni* (Thorne, 1969) Franklin, 1971 (LEITE et al., 2020), *M. javanica* (Treub, 1885) Chitwood, 1949, and *M. oryzae* Mass, Sanders and Dede, 1978 (MATTOS et al., 2017). In other studies, conducted in assays under greenhouse conditions, reproduction of *M. graminicola* has been reported in *E. crus-galli*, *C. difformis* and *C. iria* L. 1753, (good hosts) in Rio Grande do Sul and Santa Catarina (NEGRETTI et al., 2014). Furthermore, also with artificial inoculation under greenhouse conditions, *M. ottersoni* was confirmed in *E. crus-galli*, *E. colonum*, and *Phalaris canariensis* L., 1753, but little information is available about the range of hosts of this species associated with rice crops (LEITE et al., 2020).

Due to the scenario described above, this study characterized, in greenhouse conditions, the reaction of weeds associated with irrigated rice crops in relation to the species *M. ottersoni* and *M. graminicola*.

MATERIALS AND METHODS

The host suitability of weeds that occur in flooded rice fields to *M. ottersoni* and *M. graminicola* was evaluated under greenhouse conditions at Embrapa Clima Temperado, Pelotas, Brazil. Previously, we applied electrophoresis to confirm the purity of the inocula (CARNEIRO & ALMEIDA, 2001). The experiments with *M. ottersoni* were conducted from December 15, 2020, to February 26, 2021 (#1) and again from February 23, 2022, to May 4, 2022 (#2). In the case of *M. graminicola*, the experiments were carried out from December 20, 2020, to March 3, 2021 (#3) and from February 24, 2022, to May 5, 2022 (#4).

Inoculum origin and identification

Isolates were obtained from samples collected in flooded rice fields located in Capão do Leão (*M. ottersoni*) and Uruguaiana (*M. graminicola*), Rio Grande do Sul state, Brazil. The isolates (one eggs mass) were routinely multiplied on rice plants under greenhouse conditions (25 ± 5 °C). Both *Meloidogyne* species were identified based on esterase phenotypes as *M. ottersoni* (Est Ot0; Rm=0) and *M. graminicola* (Est G2; Rm: 0.85, 0.91), according to LEITE et al., (2020). For this purpose, protein extract from both nematodes were individually submitted to a horizontal (continuous) electrophoresis system with polyacrylamide gel (7%) (CARNEIRO & ALMEIDA, 2001) using *M. javanica* [Est J3 (Rm: 1.0, 1.20, 1.35)] as reference.

Weed seeds: collection, treatment, and sowing

Seeds of weeds were collected from a lowland rice field at the Palma Agricultural Center/UFPel, located at Capão do Leão, Rio Grande do Sul, Brazil. Seeds collected from *C. ferax*, *C. iria*, and *C. difformis* were submitted to thermal treatment at 40 °C for 3 days to break dormancy (DERAKHSHAN & GHEREKHLOO, 2013). Seeds with fast germination but slow emergence (Cyperaceae) were firstly sown, while seeds with slow germination and fast emergence (Poaceae) were sown later (3 days) in a commercial substrate (Germina Plant Horta Turfa Fértil®) and maintained under greenhouse conditions (25 ± 5 °C).

Experimental design

The experiments with both nematodes were performed twice under greenhouse conditions (25 ± 5 °C). In both experiments, the design was randomized blocks with 6 (#1, 2 and 3) and 5 (#4) repetitions with 10 treatments (weeds species + control). The weeds tested were *C. ferax*, *C. iria*, *C. difformis*, *Spergula arvensis* L., *O. sativa* (red rice), *E. crus-galli*, *E. colonum*, *Aeschynomene denticulata* Rudd, and *Leersia hexandra* Sw. There was only one assessment for the species *C. iria* and *C. difformis*, since the seeds did not germinate in the first and second periods, respectively.

For the experiments with *M. graminicola*, seedlings with two leaves were transplanted to pots containing 1 L of sterile substrate (18% clay). Experiments with *M. ottersoni* had seedlings with two leaves transplanted to pots with 3 L of the same sterilized substrate (18% clay). *Oryza sativa* cv. BRS Querência (*M. graminicola*) and *O. sativa* cv. IRGA 424 (*M. ottersoni*) were used as susceptible control.

Inoculation of M. ottersoni and M. graminicola and evaluation criteria

Inoculum of *M. ottersoni* and *M. graminicola* was extracted from the roots of rice

plants, according to the method proposed by HUSSEY & BARKER (1973), using a blender instead of manual shaking for 30 seconds with sodium hypochlorite solution (BONETI & FERRAZ, 1981). The suspension obtained was then poured into attached sieves and the specimens were collected on the 500-mesh sieve. After 10 days, these plants were inoculated with approximately 5,000 specimens (eggs plus J2s) (initial population - IP), with the inoculum being deposited at an approximate depth of 2 cm around each plant (two holes). Ten days after inoculation (DAI), the water level was adjusted at 1 cm above the soil and maintained during the experimental period. The plants inoculated with *M. ottersoni* were evaluated at 71 DAI (first evaluation) and 73 DAI (second evaluation), while those inoculated with *M. graminicola* were evaluated at 70 (first evaluation) and 73 DAI (second evaluation).

Evaluation of nematological variables

Plant root systems were examined regarding the number of galls (NG) and then were separated from the shooting part, washed, weighed, ground, and processed for extraction of eggs and second-stage juveniles (J2s), according to the method described by HUSSEY & BARKER (1973), using a blender instead of manual shaking for 30 seconds with sodium hypochlorite solution (BONETI & FERRAZ, 1981). The suspension obtained was then poured into attached sieves and the specimens were collected on the 500-mesh sieve. The extracted specimens (Final population – FP) were counted nematodes on Peter's slide and used to calculate the reproduction factor (RF=FP/IP), according to OOSTENBRINK (1966).

Statistical analysis

The data were analyzed using the R software (version 4.2.1) (R DEVELOPMENT CORE TEAM, 2022). The data referring to the variables NG and RF were transformed by CenterScale, $(x+1)^{1/2}$ and $(x+0.5)^{1/2}$, when necessary to satisfy the assumptions for analysis of variance (ANOVA), with the bestNormalize package version 1.8.3 (PETERSON, 2021). The Shapiro-Wilk and Bartlett tests were applied to assess the normal distribution of the residuals and homoscedasticity of the variances, respectively.

When the assumptions of ANOVA were satisfied, the data were submitted to the Scott-Knott test for comparison of the means ($P \leq 0.05$). When the assumptions were not satisfied, even after the transformations, the nonparametric Friedman test was used to analyze the data, with the separation of the means accomplished by the method of Bonferroni adjusted to a confidence interval of 0.05. We considered the weeds to be resistant (poor hosts) when plants showed RF < 1.00; susceptible (good hosts) when RF ≥ 1.00; and immune (non-hosts) with RF=0,00 (OOSTENBRINK, 1966).

RESULTS AND DISCUSSION

The reactions of weeds to *M. ottersoni* are presented in table 1. Differences between the variables were observed between treatments ($P \leq 0.05$): *A. denticulata* did not have galls in any of the evaluations, while *S. arvensis* presented only a small number in the first evaluation (0.04 ± 0.05) and *L. hexandra* presented only a small number in the second evaluation (3.17 ± 2.92) (Table 1). The species *E. colonum* presented intermediate results in both assessments (4.83 ± 0.98 and 15.83 ± 7.57). The greatest NG results were presented by *C. ferax* (12.16 ± 2.13 and 0.83 ± 0.98), *C. iria* (7.83 ± 1.47 and 30.00 ± 19.96), *E. crus-galli* (6.84 ± 2.22 and 18.17 ± 10.26) and *C. difformis* (11.00 ± 1.67). Nevertheless, in comparison with the rice cultivar IRGA 424, this number was very small for all species (129.33 ± 27.74).

With regard to RF, the lowest values were observed for *C. ferax* (0.23 ± 0.08 and 0.07 ± 0.09), *S. arvensis* (0.05 ± 0.04 and 0.00 ± 0.00), *A. denticulata* (5.46 ± 1.90 and 0.02 ± 0.03) and *L. hexandra* (3.53 ± 1.02 and 0.20 ± 0.49), which were significantly lower ($P \leq 0.005$) in comparison with *C. iria* (8.59 ± 0.76 and 29.90 ± 11.54), *E. crus-galli* (7.86 ± 0.96 and 25.22 ± 10.46) and *E. colonum* (7.26 ± 1.23 and 26.63 ± 9.62), so they were considered to be good hosts (Table 1). The *C. difformis* plants, although only evaluated once, were classified as susceptible (5.33 ± 1.74).

Despite the low NG value, *C. iria*, red rice (7.17 ± 0.75), *E. crus-galli* and *E. colonum* were classified as good hosts of *M. ottersoni* (RF > 1.0). The species *A. denticulata* (0.00 ± 0.00) and *L. hexandra* (0.00 ± 0.00) were judged susceptible in the first evaluation (RF > 1.00), while they were classified as resistant (RF < 1.00) in the second assessment. The species *C. ferax* (0.83 ± 0.98) and *S. arvensis* (0.00 ± 0.00) were considered resistant (RF < 1.00).

For *M. graminicola*, there were significant differences between the treatments regarding the variables assessed ($P \leq 0.05$) (Table 2). In the first experiment (2021), the highest NG value was observed for the cultivar BRS Querência (139.60 ± 27.67), followed by red rice (4.20 ± 0.50) and *C. difformis* (6.00 ± 0.95). No galls were detected in the other species. In the second experiment (2022),

Table 1 - Reaction of common weeds in rice fields with Meloidogyne ottersoni.

Treatments	----------NG----------				----------RF----------				Reaction[1]	
	2021[*]		2022[**]		2021[***]		2022		2021	2022
Oryza sativa 'IRGA 424'+	-		129.33	a	-		32.24	a	-	S
Cyperus ferax	12.16	a	0.83	c	0.23	d	0.07	d	R	R
Spergula arvensis	0.04	d	0.00	c	0.05	d	0.00	d	R	I
Cyperus iria	7.83	b	30.00	b	8.60	a	29.90	a	S	S
Oryza sativa (red rice)	7.17	b	19.00	b	5.13	b	12.30	c	S	S
Echinochloa crus-galli	6.83	b	18.17	b	7.86	a	25.22	b	S	S
Echinochloa colonum	4.83	c	15.83	b	7.26	a	26.63	b	S	S
Aeschynomene denticulata	0.00	d	0.00	c	5.46	b	0.02	d	S	R
Leersia hexandra	0.00	d	3.17	c	3.53	c	0.20	d	S	R
Cyperus difformis	11.00	a	-	-	5.33	b	-	-	S	-
CV%	3.89		30.04		10.53		34.49			

Means followed by the same letter in the column do not differ significantly based on the Scott-Knott test at 5%; [*] original values transformed with CenterScale, [**] (x+1)1/2 e, [***] (x+0,5)1/2; CV = Coefficient of variation; NG = Number of galls; RF = reproduction factor; Sign - = Missed treatment.+ Control.
[1]Resistance/susceptibility reaction according to OOSTENBRINK (1966). R – Resistant or poor host; S – Susceptible or good host, I – Immune or non-host.

the species with the highest NG value was 'BRS Querência' (36.8 ± 15.51), a significantly higher result ($P \leq 0.05$) in comparison with red rice (1.4 ± 1.14) and *E. crus-galli* (1.4 ± 1.67).

Although red rice and *E. crus-galli* presented low NG values, *O. sativa* was a good host to *M. graminicola* in both experiments (RF = 1.08 ± 0.09 and 5.42 ± 1.85), while *E. crus-galli* was classified as resistant in both (RF = 0.56 ± 0.05 and 0.23 ± 0.08) (Table 2). The species *C. ferax* presented higher NG in the second evaluation (18.0 ± 12.28) and variable RF (0.00 ± 0.00 and 3.76 ± 1.32), thus being classified as a good host. The other species were classified as immune or non-hosts. In this respect, the immunity/

Table 2 - Reaction of common weeds in rice fields with Meloidogyne graminicola.

Treatments	----------NG----------				----------RF----------				Reaction[1]	
	2021[*]		2022[**]		2021[*]		2022[*]		2021	2022
'BRS Querência'+	139.60	a	36.80	a	4.50	a	2.13	a	S	S
Oryza sativa (red rice)	4.20	ab	1.4	c	1.08	ab	5.42	a	S	S
Echinochloa crus-galli	0.00	b	1.4	c	0.56	ab	0.23	ab	R	R
Leersia hexandra	0.00	b	0.00	c	0.00	b	0.02	ab	I	R
Spergula arvensis	0.00	b	0.00	c	0.00	b	0.04	ab	I	R
Cyperus ferax	0.00	b	18.0	b	0.00	b	3.76	a	I	S
Aeschynomene denticulata	0.00	b	0.00	c	0.00	b	0.00	b	I	I
Echinochloa colonum	0.00	b	0.00	c	0.00	b	0.43	ab	I	R
Cyperus iria	-	-	11.80	b	-	-	0.11	ab	-	R
Cyperus difformis	6.00	ab	-		0.38	ab	-		R	-
CV %	64.81		30.40		8.11		35.73			

Means followed by the same letter in the column do not differ significantly, [*] original values analyzed with the nonparametric Friedman test and [**] original values transformed by √(x+1) and means analyzed based on the Scott-Knott test at 5%; CV = Coefficient of variation; NG=Number of galls; RF = Reproduction factor. Sign - =Plant not evaluated, +Control.
[1]Resistance/susceptibility reaction according to OOSTENBRINK (1966). R – Resistant or poor host; S – Susceptible or good host, I – Immune or non-host.

resistance of the species *L. hexandra* (RF = 0.00 ± 0.00 and 0.02 ± 0.01), *S. arvensis* (RF = 0.00 ± 0.00 and 0.04 ± 0.01), *A. denticulata* (RF = 0.00 ± 00 and 0.00 ± 0.00) and *E. colonum* (RF = 0.00 ± 0.00 and 0.43 ± 0.43) was verified, with the cultivar BRS Querência (RF = 4.50 ± 0.14 and 2.13 ± 0.60) differing.

Similar results were observed when weeds were inoculated with *M. graminicola*, where *A. denticulata*, *L. hexandra* and *S. arvensis* did not present symptoms or reproduction (NEGRETTI et al., 2014). Although those authors classified *S. arvensis* as immune, DABUR et al. (2004) considered it to be a host to the same plant-parasitic nematode.

Although the susceptibility of *O. sativa* and *E. crus-galli* to *M. graminicola* has been reported in previous studies (NEGRETTI et al., 2014; KUMAR et al., 2019), in our study *E. crus-galli* presented as poor host, while *O. sativa* was a good host. In the study carried out by NEGRETTI et al. (2014), red rice had a higher NG value (38.0) and similar RF value (3.67) in comparison with our results. The low NG and RF results of *E. crus-galli* in our study did not agree with those found by NEGRETTI et al. (2014) and SOARES et al. (2022), who also classified this species as a good host for *M. graminicola*, with high RF values in irrigated (5.4 and 110.2) and rainfed conditions (16.20 and 20.30).

We also observed differences regarding the RF values of the Poaceae species inoculated with *M. ottersoni*. We found that *E. colonum*, *E. crus-galli* and red rice were susceptible to *M. ottersoni*, corroborating the observations of LEITE et al. (2020), who reported the ability of these plants to host *M. ottersoni*, with high RF values for *E. colonum* (110.77) and *E. crus-galli* (61.56).

Regarding the sedge species evaluated, there was variation in relation to the RF of *M. graminicola* (Table 2). The species *C. iria* and *C. difformis* were only evaluated once, as resistant, unlike *C. ferax*, which was immune and susceptible in the first and second evaluations, respectively. These results differed from those described by NEGRETTI et al. (2014), who observed RF values higher than 1.0 for the first cited species. DABUR et al. (2004) also confirmed the ability of *C. iria* to host *M. graminicola*, while *C. difformis* was considered a good host since it can multiply in the plants in rice-wheat crop sequences. Likewise, for *M. ottersoni*, the susceptibility of *C. difformis* and *C. iria* was verified, but *C. ferax* was classified as resistant in both assessments.

The different host reactions found can result from intraspecific variability of the plants and/or physiological variation of the plant-parasitic nematodes (POKHAREL et al., 2010), as well as climate factors (KUMAR et al., 2021). In the case of weeds, the differences can be presumably attributed to the natural variability of the species studied. On the other hand, we could certainly theorize about the variability of RKN populations as well. SOARES et al. (2022) verified that different plants have different responses according to the plant-parasitic nematode, because when analyzing the effect of different variants of *M. graminicola* within each plant species, they observed significant differences in the most susceptible plants, among them *E. crus-galli* and *E. colonum*, with the G1 variant being most aggressive, followed by G3 and the G2 population. Indeed, some authors have also suggested the possibility that different biotypes (races) of *M. graminicola* share unique physiological traits, which can affect the reproductive capacity in specific hosts (SASSER, 1979).

Another factor that can influence the reproduction of plant-parasitic nematodes is soil temperature (ROBERTS et al., 1981) between our research and those described in literature. Studies have demonstrated low initial infection by plant-parasitic nematodes, so it is likely that the combination of low soil temperature and low reproductive potential of the plants results in little or no increase in the number of plant-parasitic nematodes during the evaluation cycle (PLOEG & MARIS, 1999; TIMPER et al., 2006). However, temperatures between 29 °C and 38 °C favor the development of plant-parasitic nematodes (DEVARAJA et al., 2022). The temperature can explain, at least partially, any discrepancies observed in our experiments, since the maximum reached in a greenhouse is 25 °C (greenhouse conditions), but the average minimum temperature in the region during the experimental period was between 17 (experiments 2 and 4) and 23 °C (experiments 1 and 3). This temperature range was slightly lower than those found in the literature specifically for *M. graminicola* (MANTELIN et al., 2017), in which some authors also report temperature ranges between 22 and 29 °C and between 27 and 37 °C (RUSINQUE et al., 2021). The variation of infection can also be associated with temperature changes (RAVINDRA et al., 2017). Our experiments were carried out in different periods when variations in the average temperatures might have influenced the life cycle of the plant-parasitic nematodes. Studies have demonstrated that the cycle of *M. graminicola* can vary from 19 to 65 days, depending on the temperature. Hence, the number of generations of plant-parasitic nematodes can differ greatly in the same vegetative cycle of the infected plant (RAVINDRA et al., 2017).

Similar results were found for the weed host status of *M. ottersoni*, where LEITE et al. (2020) found higher RF values for *E. crus-galli* and *E. colunum* at higher temperatures (15 - 25 ºC). Perhaps the lack of flooding could explain the higher RF values in this study. Unfortunately, little research has been done on this nematode. Its distribution is probably underestimated because it is difficult to detect, and few studies have been carried out on its biology.

We observed that the weeds with RF > 1.0 can act as important multiplier agents of *M. graminicola* and *M. ottersoni*. Our findings are important by contributing to knowledge of the wide range of weeds that can serve as hosts of both plant-parasitic nematodes. Therefore, these results can be utilized as tools to monitor these crop pathogens, to make recommendations for more effective management seeking to eliminate these plants through the application of herbicides or the use of cover plants to suppress plant-parasitic nematodes and minimize crop losses (RICH, 2009; JAIN et al., 2012).

CONCLUSION

Of the weed species that occur between irrigated rice crops, *S. arvensis* was found to be a poor host of *M. ottersoni*, while *L. hexandra* and *A. denticulada* are good hosts. Among the species tested, all except *C. ferax* were able to serve as hosts for plant-parasitic nematodes. The presence of these species in cropland can serve as alternative hosts, so knowledge in this respect is useful to plan measures to control nematodes and eliminate weeds.

The species *L. hexandra* and *S. arvensis* are poor hosts of *M. graminicola*, and *A. denticulada* was immune to the nematode.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Conceptualization: KJAL, CBG, DA and JVAF. Data acquisition: KJAL, DGRL and DMC. Design of methodology and data analysis: CBG and KJAL. KJAL prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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