

Productivity of main stem and tillers of rice as a function of nitrogen fertilization fractionation

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ABSTRACT: The objective of this work was to evaluate the contribution of the main stem and tillers on the grain yield of irrigated rice, and on the physiological quality of the seeds as a function of fractionation of topdressing nitrogen fertilization. The experiment was performed in the field, at the 2016-2017 and 2017-2018 growing seasons. The treatments consisted of five nitrogen (N) topdress fractionations 50% of the rate at V3, and 50% at R0; 67% at V3, and 33% at R0; 60% at V3, 20% at V6, and 20% at R0, 100% at V3, and a control without N topdress application. The main stem panicle, and each tiller class were harvested, and grain yield (GY), yield components and physiological seed quality were evaluated. Grain yield and physiological seed quality are influenced by the panicle classes, being higher in the main stem panicle. The N fractionations of 67% at V3 and 33% at R0; 60% at V3, 20% at V6 and 20% at R0 and 100% at V3, promote higher GY and number of grains per panicle. The thousand grains mass, spikelet sterility, the physiological quality of seeds are not influenced by the topdressing nitrogen fertilization fractionation.

Key words: germinating; nitrogen; *Oryza sativa* L.; tillering

Produtividade do colmo principal e perfilhos de arroz em função do fracionamento da adubação nitrogenada

RESUMO: O objetivo do trabalho foi avaliar a contribuição do colmo principal e dos perfilhos na produtividade de grãos de arroz irrigado e na qualidade fisiológica das sementes em função de fracionamentos da adubação nitrogenada em cobertura. Os tratamentos consistiram em cinco fracionamentos de nitrogênio (N) em cobertura: 50% da dose em V3 e 50% em R0; 67% em V3 e 33% em R0; 60% em V3, 20% em V6 e 20% em R0, 100% em V3 e um controle sem aplicação de N em cobertura. Foi realizada a colheita da panícula do colmo principal e de cada classe de perfilho, sendo avaliada a produtividade de grãos, os componentes do rendimento e a qualidade fisiológica das sementes. A produtividade de grãos e a qualidade fisiológica de sementes são influenciadas pelas classes de panículas, sendo maiores na panícula do colmo principal. Os manejos de N de 67% em V3 e 33% em R0; 60% em V3, 20% em V6 e 20% em R0 e 100% em V3, promovem maior produtividade de grãos e número de grãos por panícula. A massa de mil grãos, esterilidade de espiguetas e a qualidade fisiológica de sementes não são influenciadas pelos fracionamentos da adubação nitrogenada em cobertura.

Palavras-chave: germinação; nitrogênio; *Oryza sativa* L.; perfilhamento



Introduction

The rice plant (*Oryza sativa* L.), morphologically, consists of the formation of the main stem and tillers, which start at different times and differ in their growth pattern (Yoshida, 1981). A difference can be found in the time of panicle emission and grain development in panicles of the same plant. Thus, grain yield and yield components vary with the timing of tiller emergence, with tillers emitted earlier in the plant tending to have higher grain yields compared to those emitted later (Wang et al., 2016).

High tillering capacity is considered a desirable trait in rice plants, as the number of tillers is related to the number of panicles per plant (Zhu et al., 2019), and is the yield component with the greatest influence on crop grain yield (Fageria et al., 2013). However, plants with a greater number of tillers exhibit disuniformity in the distribution of nutrients and photoassimilates among tillers, resulting in variation in grain development and yield among tillers (Wang et al., 2016). In addition, seed physiological quality is also influenced by tillering ability, because the time elapsed between flowering and harvest in panicles emitted later by the plant is reduced, resulting in a lower degree of maturation and reduced seed physiological quality (Smiderle & Pereira, 2008).

Several factors interfere with tiller formation and development in flood irrigated rice, including water blade height (Zhu et al., 2019), water temperature (Liu et al., 2019), radiation incidence on the basal buds (Wang et al., 2016), plant density and nutrient availability (Yoshida, 1981), among others. Regarding nutrition, N is one of the most important nutrients for the rice plant, standing out as the most limiting to grain yield (Santos et al., 2017; Fidelis et al., 2018; Qi et al., 2020) and being directly related to plant tillage capacity (Wang et al., 2017; Liu et al., 2019).

N is associated with several physiological processes in the plant, such as the formation of the chlorophyll molecule, enzymes, proteins, and nucleic acids, and is thus essential for its proper growth and development (Kishorekumar et al., 2020). In addition, it affects yield components such as panicle number, number of grains per panicle, thousand-grain mass, and promotes reduced spikelet sterility, resulting in higher grain yield (Fageria et al., 2013). However, the losses of this nutrient in the soil-water-plant system are high, and the recovery of the N applied may be less than 50%, highlighting in these losses the processes of volatilization, leaching and denitrification (Santos et al., 2017; Shi et al., 2020).

Nitrogen fertilizer fractionation is an important tool in mitigating the loss processes of this nutrient (Liang et al., 2017; Fidelis et al., 2018). In this sense, it must meet the plant's requirements at important moments in the definition of the crop's yield components, especially the period between the start of tillering and the reproductive phase of the plants. For the Sociedade Sul Brasileira de Arroz Irrigado (SOSBAI), the fractioning should be done as 2/3 of the nutrient before the definitive water entry into the field, V3/V4 stage (Counce et al., 2000) and 1/3 at panicle initiation, R0 stage (panicle

initiation). However, doubts are still frequent regarding the proportion of fractioning and the timing of the application of nitrogen fertilizer coverage, in order to provide greater synchronism between the availability of the nutrient in the soil and the demand by the plant. Among the variables that can affect the efficiency of assimilation of N made available via fertilizer are sowing time, plant development stage, and variety cycle (SOSBAI, 2018).

The total supply of nitrogen fertilizer in cover at the V3/V4 stage enables greater availability of the nutrient in the soil and in the solution at the time of great demand for tiller formation, however, it concentrates a large amount of the nutrient at a stage when the plant has little assimilation capacity, increasing the chances of loss of the nutrient in the soil-water-plant system (Aramburu, 2018). Fractionation in two or more applications, on the other hand, despite promoting greater synchronism between the availability of N in the soil and the demand by the plant, results in increased production cost, as well as the possibility of losses due to the application of the nutrient under water blade (Aramburu, 2018).

In view of the above, the present study aimed to evaluate the contribution of the main stem and tillers on the grain yield of flood irrigated rice and on the physiological quality of seeds as a function of nitrogen fertilizer fractions.

Materials and Methods

The experiment was conducted under field conditions during the 2016/17 and 2017/18 agricultural seasons, in a systematized floodplain area belonging to the Department of Plant Science of the Universidade Federal de Santa Maria (UFSM), at different locations from the experimental area in the two crop years. The soil of the experiment is classified as Planossolo Háplico Eutrófico arênico, belonging to the mapping unit Vacacaí (Santos et al., 2018) and contained the following physicochemical attributes at 60 days before sowing in the 0-0.2 m depth layer: clay = 27%; OM = 2.0%; $\text{pH}_{\text{water}} (1:1) = 5.6$; base saturation = 53.4%; Al saturation = 2.7%; P-Mehlich = 6.0 mg dm⁻³; K = 54.0 mg dm⁻³; and, CTC pH7 = 16.1 cmol_c dm⁻³ (2016/17 crop), and clay = 28.0%; OM = 2.3%; $\text{pH}_{\text{water}} (1:1) = 5.6$; base saturation = 63.1%; Al saturation = 2.7%; P-Mehlich = 6.0 mg dm⁻³; K = 54.0 mg dm⁻³; and, CTC pH7 = 16.1 cmol_c dm⁻³ (2017/18 crop).

The experiment was set up in a randomized block design with four repetitions. The treatments were composed of nitrogen fertilizer fractions at different phenological stages of the crop: T1 - Witness, no N cover application; T2 - Fractionation of 50% of the dose at V3 (Counce et al., 2000) and 50% at R0; T3 - Fractionation of 67% of the dose at V3 and 33% at R0; T4 - Fractionation of 60% of the dose at V3, 20% at V6 and 20% at R0; T5 - 100% of the dose at V3, total supply of fertilizer coverage at the V3 stage.

Sowing was performed on November 06, 2016 and October 06, 2017. The rice cultivar used was IRGA 424 RI, at a density of 80 kg of seeds per hectare. Fertilization at sowing was composed of 16 kg ha⁻¹ of N, 68 kg ha⁻¹ of P₂O₅, and 108

kg ha⁻¹ of K₂O. For the definition of the nitrogen fertilization in cover, the soil organic matter content (SOM) and high expected response to fertilization were considered, totaling a dose of 150 kg ha⁻¹ of N. Urea (45-00-00) was used as the source and nitrogen fertilization at the V3 stage was performed on dry soil, and definitive irrigation of the area was started immediately after fertilization. The nitrogen fertilization in the V6 and R0 stages was done under water, which was maintained until the R7 stage, and then irrigation was suppressed in the experimental units. The cultural treatments were carried out according to the technical recommendations for irrigated rice culture (SOSBAI, 2018).

The experimental units were composed of 27 sowing lines spaced at 0.17 m, with a length of 6 m, totaling a useful area of 27.54 m². At the V3 phenological stage, before the start of irrigation, the main canes of 20 plants in sequence in each experimental unit were marked with colored wires. The evolution of the phenological stages of plant development was monitored, identifying the new stalks issued. In the 2016-2017 crop, the main stalk and the stalks of the first and second tillers were identified. In the 2017-2018 crop, in addition to identifying the main stalk and the stalks of the first and second tillers, the remaining stalks were identified when emitted by the plant, and these were classified as remaining stalks. At the physiological maturity of the crop (R9 stage) the panicles were harvested separately and grouped according to their class. The yield components: number of grains per panicle, thousand-grain mass, spikelet sterility, and grain yield per panicle were evaluated.

The germination test was performed using four repetitions of 100 seeds per treatment, sown on paper rolls with two leaves, of the germitest type, previously moistened with distilled water at 2.5 times the weight of the paper. The rolls were placed in the germinator at a temperature of 25 ± 2 °C. The evaluation was performed at 14 days after sowing (Brasil, 2009). The first germination count was performed together with the germination test, computing the average percentages of normal seedlings five days after the test was installed. The results were expressed as a percentage of germinated normal seedlings.

The variables evaluated were analyzed as a two-factor (N fractionations × panicle classes). The results were submitted to the mathematical model assumptions test (normality and homogeneity of variances). Analysis of variance (ANOVA) was performed using the F test. The means of the factors, when significant, were compared by the Scott-Knott test at 5% probability of error. The first count germination and seed germination data were transformed by the equation arcsine x/100. The averages of the factors, when significant, were compared by the Scott-Knott test at 5% probability of error.

Results and Discussion

There was no interaction of the factors N fractionation × panicle classes for the variables analyzed in both seasons of the experiment. Among the panicle classes, the highest

grain yield value obtained was in the main-stem panicle, with values of 2.60 and 2.63 g per panicle in the 2016-2017 and 2017-2018 crops (Table 1), representing 45 and 37% of the total plant yield, respectively.

It can be seen that as the plant emitted new tillers there was a significant reduction in grain yield per panicle (Table 1). The panicle of the first tiller obtained values of 1.89 and 1.96 g panicle⁻¹ in the 2016-2017 and 2017-2018 crops, representing 32 and 28% of the total plant production, respectively. The second tiller panicle, on the other hand, obtained grain yield values of 1.33 and 1.65 g panicle⁻¹ in the 2016-2017 and 2017-2018 crops, respectively, contributing 23% of the total plant yield in both crops.

The remaining panicle class had the lowest expression in total plant yield, with a value of 0.84 g per panicle in the 2017-2018 crop, contributing only 12% of total plant yield. From the results one can infer that about 90% of the grain yield is found in the main stem, first and second tillers. Similar results were obtained by Wang et al. (2017), who, evaluating the participation of the main stem and tillers in the yield of irrigated rice as a function of nitrogen doses, found that the main stem panicle showed the highest grain yield, with a reduction in the contribution of each panicle class to the total plant yield as new stalks were emitted, even with higher doses of N. According to the authors, the lower grain yield of panicle classes is related to the lower number of grains per panicle and the higher sterility of spikelets, as verified for the present study.

Several physiological aspects have been reported to cause the lower productivity of later emerging tillers in plants; such as shorter duration of their growth and tolerance to photo-oxidative stress (Mohapatra & Kariali, 2008), lower leaf N concentration (Wang et al., 2017), and disuniformed distribution of photosynthetically active radiation among tillers (Wang et al., 2016).

The main stem panicle was the one that obtained the highest values of number of grains per panicle and the lowest values of spikelet sterility in both crops (Table 1). The number of grains per panicle decreased and spikelet sterility increased as the plant emitted new tillers, and these variables are directly related to the yield components of irrigated rice (Yoshida, 1981). In this sense, tillers produced later by the plant produced panicles with higher spikelet sterility and lower number of grains, possessing less competitive ability compared to panicles produced earlier by the plant, resulting in lower grain yields.

Grain yield per panicle was influenced by nitrogen fertilization fractions, with 67% V3 + 33% R0, 60% V3 + 20% V6 + 20% R0 and 100% V3 providing the highest values in both crops, with averages of 2.07, 2.12, and 2.06 g panicle⁻¹ in the 2016/17 crop and 1.91, 1.93, and 1.86 g panicle⁻¹ in the 2017/18 crop, respectively (Table 1). It can be seen that as the amount of N applied in the initial phase of plant development increased, a greater number of grains and productivity per panicle were obtained, possibly related to a greater utilization of the N applied. These results corroborate those obtained

Table 1. Number of grains per panicle (NGP), mass of thousand grains (MMG), spikelet sterility (SS) and grain yield (GY) of panicles of the main stem, primary tiller, secondary tiller and remaining plants of rice cultivar IRGA 424 RI submitted to nitrogen fertilization fractions in cover in the 2016/17 and 2017/18 crops. Santa Maria, RS, Brazil, 2018.

2016/17 crop				
Nitrogen fractionations	Variables analyzed			
	NGP	MMG (g)	SS (%)	GY (g panicle ⁻¹)
Witness	61.70 c ⁽¹⁾	25.47 ^{ns}	11.18 ^{ns}	1.56 c
50% V3 + 50% R0	74.58 b	25.44	11.21	1.89 b
67% V3 + 33% R0	80.82 a	25.52	10.94	2.07 a
60% V3 + 20% V6 + 20% R0	82.96 a	25.50	11.09	2.12 a
100% V3	80.60 a	25.50	11.26	2.06 a
Panicle classes				
Main stem panicle	100.26 a	25.52 ^{ns}	9.86 c	2.60 a
Primary tiller panicle	74.47 b	25.49	10.13 b	1.89 b
Secondary tiller panicle	53.67 c	25.45	13.42 a	1.33 c
CV%	8.87	1.36	15.64	7.34
2017/18 crop				
Nitrogen fractionations	Variables analyzed			
	NGP	MMG (g)	NGP	RG (g panicle ⁻¹)
Witness	56.99 c	25.32 ^{ns}	10.24 ^{ns}	1.43 c
50% V3 + 50% R0	69.59 b	25.37	10.05	1.74 b
67% V3 + 33% R0	74.20 a	25.40	9.51	1.91 a
60% V3 + 20% V6 + 20% R0	76.91 a	25.42	8.99	1.93 a
100% V3	72.64 a	25.42	9.57	1.86 a
Panicle classes				
Main stem panicle	103.23 a	25.47 ^{ns}	6.88 c	2.63 a
Primary tiller panicle	77.03 b	25.37	9.71 b	1.96 b
Secondary tiller panicle	65.23 c	25.44	10.52 b	1.65 c
Remaining panicles	34.29 d	25.26	11.60 a	0.84 d
CV%	8.01	1.38	13.99	7.77

^{ns} Not significant. ⁽¹⁾ Averages followed by the same letter in the column do not differ by Scott Knott test at 5% probability level.

by [Fidelis et al. \(2018\)](#), who evaluating fractions of nitrogen fertilization in twelve irrigated rice cultivars found no influence on grain yield. However, these authors found a trend toward higher grain yields when the highest dose was supplied prior to the start of irrigation at the V3 stage.

The results in the present study are related, in part, to the rapid formation of the water sheet after the application of nitrogen fertilizer at the V3 stage, which occurred within 3 hours after application, enabling its incorporation into the soil and the reduction of N losses in the form of NH₃ volatilization. According to [Dempsey et al. \(2017\)](#), after application, urea is rapidly hydrolyzed by the enzyme urease to ammonium carbonate ((NH₄)₂CO₃), causing the pH around the granule to increase and can cause significant N losses through NH₃ volatilization. In this sense, [Norman et al. \(2009\)](#), evaluating the effects of delayed irrigation after the application of different sources of nitrogen fertilizers on N losses by NH₃ volatilization, found that delaying irrigation by five days caused a 21% loss of N applied via urea in the form of NH₃ volatilization, which could be even higher depending on the soil and climate conditions of cultivation. Therefore, after the application of nitrogen fertilizer, it is essential to perform the formation of the irrigation blade within three days, in order to obtain the greatest use of the fertilizer and promote the maximization of the crop's productive potential ([SOSBAI, 2018](#)).

Another factor that may have contributed to the maximization of yield potential in the treatments 67% V3

+ 33% R0, 60% V3 + 20% V6 + 20% R0, and 100% V3 is the reduction in losses of this nutrient by NH₃ volatilization, due to the lower proportion of nitrogen fertilizer applied in coverage (on water blade) at the V6 and R0 stages. In this sense, the proportion of 50% V3 + 50% R0 fractionation may have caused nutrient restriction to the plant in the vegetative phase, due to the lower dose of N applied prior to the entry of water into the tillage in the early stages of development. Moreover, the application of the highest N dose on top of the water slide may have intensified N losses by NH₃ volatilization, restricting the availability of N to the plants at the time of flower primordium differentiation (R1 stage), reducing the number of grains per panicle and grain yield of the crop, i.e., it can be seen that this fractioning proportion does not constitute an adequate management of nitrogen fertilizer coverage, since it promotes a reduction in the number of grains and grain yield per panicle.

The thousand-grain mass variable was not influenced by the different panicle classes and by the fractions of nitrogen fertilization in the analyzed crops ([Table 1](#)). The thousand-grain mass is a stable varietal character, because grain size is physically limited by the characteristics of the lemma and papillae, having a greater influence of genetic factors ([Yoshida, 1981](#)).

Spikelet sterility was not influenced by nitrogen fertilizer fractions, however, it differed statistically for the different panicle classes ([Table 1](#)). The lowest sterility values were seen in the main stem panicle, increasing as the plant emitted new

tillers. This is directly related to the lower grain yield obtained by panicle classes as the plant issued new tillers, as spikelet sterility is considered one of the primary components of grain yield in rice (Fageria et al., 2013).

The fractions of nitrogen fertilization did not affect the physiological quality of the seeds produced (Table 2). However, panicle class affected seed quality, where the main stem panicle obtained a higher percentage of germinated seedlings in the first-count germination and seed germination evaluations in both crops. The lowest values for these variables were observed in the panicle of the secondary tiller in the 2016-2017 crop, and in the panicles of the secondary tiller and the other tillers emitted by the plant in the 2017-2018 crop, which did not differ statistically from each other. In the present study, it is verified that the youngest tillers, even producing seeds with lower germination potential, present physiological quality within the standards stipulated in Annex III of Normative Instruction 45/2013 (Brasil, 2013).

The difference in seed physiological quality between panicle classes may be related to the length of the grain filling period, considering that tiller emission occurred at different periods of the plant's vegetative development. According to Yoshida

(1981), the first tiller of a rice plant appears in the axil of the third leaf below the one expanding in the main stem, starting from the emission of the fifth leaf. This emergence of tillers occurs synchronously with the emission of new leaves by the mother plant. Taking into consideration that the period between the emission of two leaves in the main stem is approximately five days during the vegetative phase (Yoshida, 1981), it can be inferred that the emergence of the primary tiller occurred close to the emission of the fifth leaf, between the V3 and V4 stages of the main stem plant, and the panicle of the secondary tiller from 3 to 9 days after the panicle of the main stem. The panicles that emerged first contain longer grain filling period, which according to Mohapatra & Kariali (2008) is an advantage in terms of competition for light with later emerged tillers that get shaded with early aging of leaf area (Wang et al., 2016). However, even with a longer grain filling period, there is no statistical difference for the thousand-grain mass.

In general, the main-stem panicle obtained the highest number of grains per panicle, the least spikelet sterility, the highest grain yield and physiological seed quality. Thus, the results show that an interesting strategy would be to adopt tillage management practices, such as area preparation, seed quality, sowing, sowing machine, fertilization, irrigation timing and uniformity, so that tillers establish themselves as quickly as possible, reducing unevenness and the appearance of late tillers that contribute little to productivity.

As for the fractionation of nitrogen fertilizer, these had isolated effects for the variables analyzed. It was found that the application of the highest proportions of fractionation before the beginning of irrigation at the V3 stage, by the treatments 67% V3 + 33% R0, 60% V3 + 20% V6 + 20% R0 and 100% V3, provided greater number of grains and yield per panicle, maximizing the productive potential of the crop.

However, these results are possibly related to the conditions of the experiments, since the rapid formation of the water sheet after the application of treatments, which may have provided a better use of nitrogen fertilizer by the plant and reduced N losses through the NH₃ volatilization process.

Although the results signaled that applying 100% N at the V3 stage did not provide a difference in grain yield when compared to the 67% V3 + 33% R0 and 60% V3 + 20% V6 + 20% R0 fractions, this result should be interpreted with parsimony. The 100% V3 treatment shows itself as an alternative when the producer has total control over his irrigation system and the management of the crop, in conditions of sowing within the recommended time, efficient weed control, and expectation of favorable weather conditions for nutrient absorption by the crop (solar radiation). Besides this, the difficulties of applying fertilizer in only one operation should be noted, given the high volume to be applied and the need to distribute it evenly throughout the field. In this sense, due to the risks of losses and the non-assimilation of N by the plant, the total supply of nitrogen fertilization at the V3 stage is not recommended by SOSBAI (2018). Thus, to minimize the risks inherent in N losses in the soil-water-plant system and maximize the yield potential of rice, the 67% V3 + 33% R0 and 60% V3 + 20% V6 + 20% R0 fractions are viable options in irrigated rice production areas.

Table 2. First count of germination and seed germination of panicles of the main stem, primary, secondary and remaining tillers of rice plants of IRGA 424 RI cultivar submitted to fractions of nitrogen fertilization in cover in the 2016/17 and 2017/18 crops. Santa Maria, RS, Brazil, 2018.

2016/17 crop		
Nitrogen fractionations	Variables analyzed	
	First germination count (%)	Germination (%)
Witness	80.00 ^{ns}	95.33 ^{ns}
50% V3 + 50% R0	81.67	96.00
67% V3 + 33% R0	80.83	95.00
60% V3 + 20% V6 + 20% R0	81.00	95.83
100% V3	79.83	95.16
Panicle classes		
Main stem panicle	84.90 a ⁽¹⁾	98.00 a
Primary tiller panicle	80.60 b	95.00 b
Secondary tiller panicle	76.50 c	93.40 c
CV%	2.43	2.57
2017/18 crop		
Nitrogen fractionations	Variables analyzed	
	First germination count (%)	Germination (%)
Witness	77.37 ^{ns}	94.87 ^{ns}
50% V3 + 50% R0	77.50	95.37
67% V3 + 33% R0	76.75	94.62
60% V3 + 20% V6 + 20% R0	77.62	95.12
100% V3	76.50	94.50
Panicle classes		
Main stem panicle	81.10 a	97.50 a
Primary tiller panicle	78.20 b	95.40 b
Secondary tiller panicle	74.40 c	93.00 c
Remaining panicles	74.90 c	93.70 c
CV%	2.78	2.80

^{ns} Not significant. ⁽¹⁾ Averages followed by the same letter in the column do not differ by Scott Knott test at 5% probability level.

Associated with the rapid irrigation after the application of fertilizer, another factor that may have contributed to the results obtained in the experiment is the high dose of N in cover used, which may have attenuated the effects of fractionation. In this sense, under conditions of lower N doses, the effects of N fertilizer fractionation may be more pronounced, due to the need to promote greater synchronism between N availability and demand by the plant.

Conclusions

Grain yield and seed physiological quality are influenced by panicle classes, being highest in the main stem panicle.

The nitrogen managements 67% at V3 and 33% at R0; 60% at V3, 20% at V6 and 20% at R0 and 100% at V3, promote higher grain yield and number of grains per panicle.

The thousand-grain mass, spikelet sterility, and physiological seed quality are not influenced by the fractions of nitrogen fertilizer cover.

Compliance with Ethical Standards

Author contributions: Conceptualization: BBA, EM; Data curation: AGF, MHP, ADS, USR, MMW; Formal analysis: BBA, AGF, LLC; Investigation: BBA, AGF, ADS, MHP, USR, MMW; Methodology: BBA, EM; Project administration: BBA, EM; Resources: BBA, EM, AGF; Supervision: BBA, EM; Validation: BBA, EM, AGF, LLC; Visualization: BBA, AGF, LLC; Writing – original draft: BBA, AGF; Writing – review & editing: EM, LLC.

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