

Effect of Rates and Sources of N Fertilizer Application on Dynamics of Rice Brown Leaf Spot Disease (*Bipolaris oryzae*) Incidences in the Dry Zone of Sri Lanka

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Abstract

Nitrogen inputs; sources or application amounts are key determinants of yield determination and determination of resistance or sensitivity to pathogen activities. This study aimed at assessing the impact of source and rate of application of N fertilizers on Rice Brown Leaf Spot (RBLs) disease incidences and dynamics in lowland irrigated rice crops. Leaf N using relative leaf chlorophyll content, leaf N concentration and crop yield were assessed during the wet 2018/19, 2019/20 and 2020/21 seasons and dry 2019 and 2020 seasons in the field research facility of Rajarata University of Sri Lanka. The conventional systems (The Department of Agriculture recommended inorganic fertilizer application at 100% N), integrated system (50% N with conventional through inorganic fertilizer and 25% N with organic manure mixture), and organic system (50% of N conventional through organic manure) were tested using a new improved rice variety Bg300, using a randomized complete block design with six replicates. Wet and dry seasons were contrastingly different in disease prevalence, where critical levels of incidences were visible earlier in the wet season compared to the dry season. Initial stages of the study, organic systems resulted in higher disease incidences, thus reaching infections of the full crop before conventional and integrated. Several seasons of continuous organic manure incorporation enhanced the resistance of organic systems to RBLs disease compared to the rest. The leaf N concentrations were higher in conventional, thus the RBLs incidences were relatively low, due to negative correlations between disease incidences. Rice yields also resulted in a significant negative correlation with disease incidences and were diminished in integrated and organic systems later. The yield suppression due to diseases such as RBLs in organic transition can be overcome by using an integrated approach

and building a balanced substitutable nutrient management strategy.

Keywords: conventional, nitrogen fertilizer, organic, rice brown leaf spot

Introduction

Rice brown leaf spot (RBLs), also called *Bipolaris oryzae*, is a worldwide known plant epidemic affecting rice harvest loss caused by necrotrophic rice leaf fungus *Cochliobolus miyabeanus* (Webster and Gunnell, 1992). The rice brown spot infects all parts of the rice plant and symptoms mainly appeared on the coleoptile, leaf sheath, leaf blade, glumes, and spikelet leading to a 6-90 % decrease in rice yield (Padmanabhan, 1973; Webster and Gunnell, 1992; Mew and Gonzales, 2002; Imran et al., 2020). Rice brown leaf spot causes a loss of 5%- 50% yield across all lowland rice production in South and Southeast Asia. It also affects the quality and the number of grains per panicle and reduces the kernel weight (Hossain et al., 2014). Therefore, proper control of RBLs disease is very important to obtain the desired quantity and quality of grain yield.

Mineral nutrition is an important component in all cropping systems to obtain proper quality rice yield output from the crop (Roberts, 2008). N element has long been recognized as they have been associated with the size, quality, and yield of crops and with changes in disease incidence levels (Palti, 1981; Rush et al., 1997; Dordas, 2008). Depending on the amount of N applied, low-level N application was associated with increased disease risk, and high-level N application was linked with decreased disease incidence (Sun et al., 2020). It emphasized the need for an appropriate level of N management with

suitable inputs to effectively manage plant diseases.

There are different types of fertilizer management based on different sources due to availability and mineral composition. Inorganic and organic sources are classified as basic types used in Sri Lanka. The presence of the mineral element, especially N, caused changes in the growth and physiology of the crop. It also affects the development of resistance and susceptibility to the pathogen's activity. N induced resistance with the suppression of lesion enlargement (Ohata et al., 1972) while with deficiency of N decrease the resistance to rice BLS (Ou, 1985). Apart from the N level, other chemical composition of various fertilizers can promote or inhibit pathogen reactions. Therefore, the source of the N is also important for assessing the dynamics of RBLs disease. Our study was conducted to assess the effect of rates and sources of N fertilizer on dynamics of RBLs disease incidences and RBLs disease dynamics with yield.

Materials and Methods

The experiment was conducted on the farm premises of the Faculty of Agriculture, the Rajarata University of Sri Lanka at Wet (Major/Maha) 2018/19, 2019/20 and 2020/21 seasons (from November to March) Dry (Minor/Yala) 2019 and 2020 seasons (from May to September) as a part of a long-term research project. The site was located at Puliyankulama in the Anuradhapura district belongs to the agro-ecological region of DL1b (Based on National Soil Maps (EUDASM)). The study area was on imperfectly drained reddish-brown earth soils (Wickramasinghe et al., 2021). Seasonal rainfall across five seasons was 364.6 mm, 181.6 mm, 962 mm, 621.7 mm, and 751.1 mm; the mean monthly maximum and minimum temperatures were 32.32 °C and 24.14 °C, respectively (Table 1).

Field Experimental Design and Treatments

The experiment consisted of three main cropping systems which were, T₁: *Conventional system/CONV* – 100% N fertilizer applied with inorganic fertilizer application as recommended by the Department of Agriculture (DOA) 2013, T₂: *Integrated system/INT* – 75% N fertilizer applied based on 50% inorganic and 25% organic fertilizer application, T₃: *Organic system/ORG* – 50% N fertilizer applied based on organic fertilizer, and it was managed through pre-determined organic manure application. The three treatments were established as a randomized complete block design with six replicates. The plot size was 15 m × 6 m. The cropping systems were defined based on the elemental N supply and the sources (Table 2).

Therefore, the phosphorus and potassium rates were not standardized. The amount of these two elements depended on the quality of materials used to supply N in both integrated and organic cropping systems.

Crop Establishment and Management

Pre-germinated seeds of the Bg300 rice variety, a widely grown variety in dry zone of Sri Lanka, were broadcasted at a rate of 120 kg per ha in both wet season and dry season. Bg300 is not resistance to the RBLs disease. The application of inorganic fertilizer was based on the Department of Agriculture, Sri Lanka recommendation in 2013. Organic fertilizer was applied with basal dressing and at the third top dressing of inorganic fertilizers. Irrigation was carried out one week after the seed sowing and a water depth was maintained above the soil level to retain sufficient moisture throughout the cultivation period.

Pest and Weed Management

Chemicals were not applied to control insects and diseases. Weed management was carried out in CONV and INT system using herbicides (Table 3). Weeds in the ORG system of the paddy field were controlled by maintaining a standing water level and manual weeding.

Data Collection

The incidence of RBLs was recorded commencing from the time of appearance of disease symptoms at three days sampling intervals to the harvesting stage. A 50 cm x 50 cm quadrat was used in collecting samples. Different two random quadrat samples were selected from each plot. The number of infected plants in each quadrat sample was counted. The disease incidence was calculated using the following formula (Groth et al., 1999).

$$\text{Disease incidence} = \frac{\text{Number of infected plants per quadrat}}{\text{Total number of plants per quadrat}}$$

The rice harvest was threshed by hand and oven-dried at 60°C until constant weight to measure the final grain yield. Relative leaf chlorophyll content (RLC) was measured as SPAD (Soil Plant Analysis Development) value using SPAD-502-leaf chlorophyll meter (Konica Minolta Sensing Inc, Japan) at the panicle initiation stage and heading stage of rice. Total leaf N was determined by the Kjeldahl distillation procedure (Bremner and Mulvaney, 1982) at the panicle initiation and heading stages of the rice crop.

Statistical Analysis

Data from five seasons were analyzed to identify the impact of different cropping systems on the RBLS disease incidences. Data were plotted using origin software. Count data were tested for normality and heteroscedasticity by identifying the seasons, then the data were log-transformed to maintain the homogeneity and normality of residuals. Analysis of variance (ANOVA) was carried out using the Repeated measures MIXED model using SAS 9.0 software to determine the effect of cropping systems, cropping season and crop stage on crop yield, relative leaf chlorophyll content and leaf N content. The season was considered the repeated factor. The means were separated using the Least significant difference (LSD) method at the 5% probability level. The correlation between RBLS disease incidences with rice crop yield, relative leaf chlorophyll content and leaf N (at the heading stage) was assessed using Pearson correlation coefficients at a 5% probability level.

Results and Discussion

Rice brown leaf spot disease was reported in both wet seasons and dry seasons. The wet season showed a significantly ($P < 0.05$) 29% greater disease incidences than the dry season, where on the dry season, the incidences were substantially low (Figures 1 and 2). The pathogen development was accelerated with regular wet conditions under higher rainfall and relative humidity during the wet season compared to the dry season (Table 1). Wickramasinghe et al., 2021 also expressed the same behavior of pathogens (narrow brown spot disease) during the wet season in the same agro-ecological zone. The disease symptoms were aroused in a different stage of the crop linked to the conditions of the growing environment. The disease was recognizable 48 days after sowing (DAS) in the wet seasons and was identified to vary late in the dry seasons. During the dry season, the incidences were noticeable near the dough stage of the rice crop/81 DAS onward, while in

wet seasons, the incidences were robust during the vegetative growth even before the anthesis (Table 5).

The disease incidences had increased over time during the growing season, while the disease progression pattern was different for three cropping systems. The wet 2018/2019 season (Figure 1) resulted in a different disease progression compared to the other three seasons. During the wet 2018/2019 season, incidences were recorded 10–20 days than the other seasons. The incidences of RBLS started to appear at or during the reproductive stage of the rice crop, yet in the dry seasons, it was delayed till the latter part of the reproductive stage (Figure 1). The observations were very similar to the findings of Kohls et al. (1987), accordingly, the RBLS epidemic began in the rice crop during the reproductive stage. Jha (2001) and Sunder et al., (2014) also reported rice plants are more susceptible from panicle formation to the dough and mature stages, where large spots were developed during this study too. The inherent resistant mechanisms might be able to resist the pathogen during the vegetative phase, however, the biochemical and physiological transition of the crop during anthesis let the crop weaken the defense for focusing on yield formation. Thus, even during dryer seasons, the incidences were possible after anthesis. The disease susceptibility might be low during the initial growth phase of the crop in both dry seasons linked to low pathogenic activities (Figure 2) due to the dryness of the growing conditions. The pathogen might have overcome the defenses with the crop senescence, thereby the disease incidences were prominent (Ou, 1985).

The leaf N concentration is the most critical parameter that governs the development of disease resistance or risks. The leaf N and relative leaf chlorophyll content of conventional, integrated, and organic systems differed in descending order. The organic cropping system results in substantially low leaf N contents. Reflectively, the disease incidences percentage and AUDPC values were higher in organic compared to conventional and integrated systems (Figures 1, 2 and Table 6), irrespective of the season. This inverse

Table 1. Values of cumulative seasonal rainfall, minimum and maximum temperatures and day and night relative humidity were recorded in the dry 2019, 2020 seasons and wet 2018/19, 2019/2020, 2020/2021 seasons.

	Wet 2018/19	Dry 2019	Wet 2019/20	Dry 2020	Wet 2020/21
Cumulative Seasonal Rainfall (mm)	364.6	181.6	962	621.7	751.1
Minimum T (°C)	23.4	25.4	23.4	25.1	23.4
Maximum T (°C)	32.4	34.1	31.6	32.7	30.8
Day RH (%)	67.6	64.6	71.2	70.4	74.8
Night RH (%)	90.6	87.4	91.8	89.2	93.1

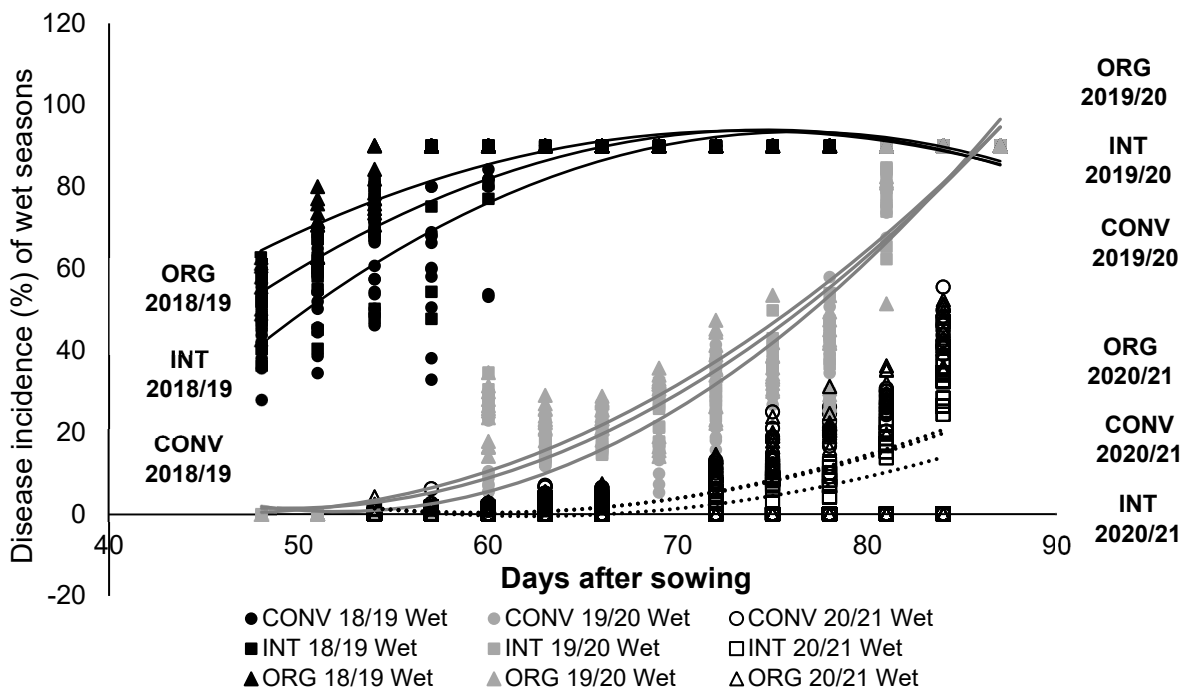


Figure 1. Rice brown leaf spot disease incidences under different cropping systems in 2018/19, 2019/20 and 2020/21 wet seasons

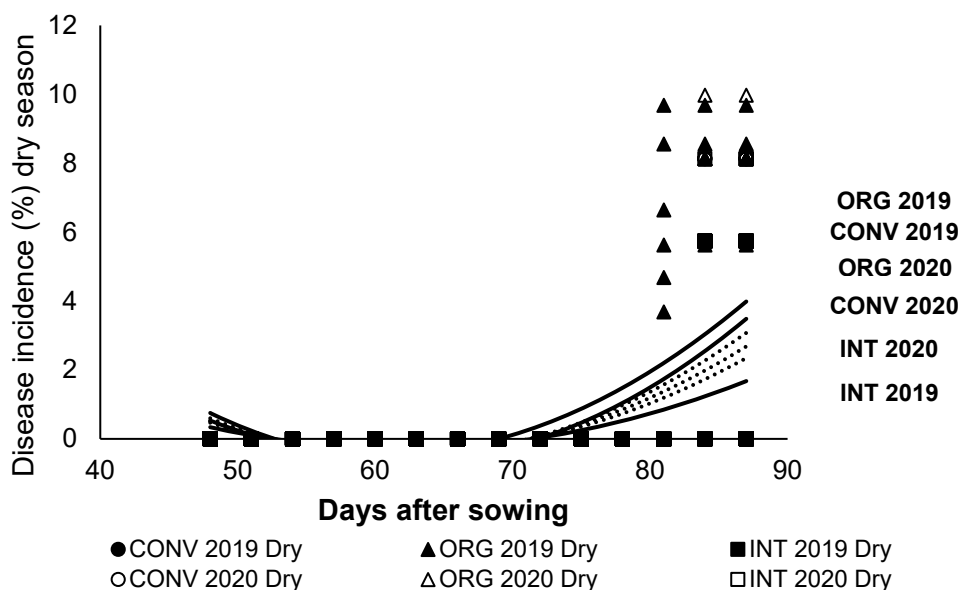


Figure 1. Rice brown leaf spot disease incidences under different cropping systems in 2018/19, 2019/20 and 2020/21 wet seasons

relationship of lower disease incidences with high N was also reflected through the correlations; the higher N content led to a decrease the disease incidences even at the heading stage (Table 4).

In wet seasons, the correlations between leaf N concentrations and disease incidences were negative and robust irrespective of the year. The relative leaf chlorophyll content showed a significant negative correlation with disease incidences only in the wet

2019/20 season (Table 5). The provenance of the disease incidences was high in organic followed by integrated and conventional at 48 DAS, in the wet season (2018/19), which showed a perfect inverse relationship to leaf N and relative leaf chlorophyll content at the panicle initiation stage. The disease susceptibility showed a clear difference with respect to leaf N concentration at the panicle initiation stage, compared to other seasons. However, the changes in reproductive to the grain filling and the possible

reduction of defense mechanisms allowed the disease to progress irrespective of the growing condition, leaf N level, or the source of N delivery.

It was clear during the wet 2019/20 and 2020/21 seasons that the high leaf N concentration at the panicle initiation stage was linked to the delay in disease progression compared to the initial wet season (2018/19) (Figure 1 and Table 5). This might be associated with the uptake efficiency of the crop with prevailed climatic and edaphic conditions. Commonly, the correlations stated negative despite the non-significance in wet 2019/20 and 2020/21 seasons illustrating the effect of N level on the disease incidence. However, no correlations between relative leaf chlorophyll content and disease incidences and leaf N concentration and disease incidences were observed in both dry seasons due to the absence of the disease incidences at panicle initiation and heading stage of the crop (Figure 2 and Table 5).

The N input was low in the organic system (50% of total N of conventional), resulting in increased disease risk, the high-level N application of the conventional system was low in disease incidences. The Intermediate N level of the integrated system (75%

of total N of conventional) resulted in an intermediate disease incidence. Hence, an appropriate level of N with the correct source of inputs can barricade the disease from development and spread. The greenness or the chlorophyll concentration within leaf tissues might be able to resist lesion enlargement by compensating for the tissue loss due to the disease (Ohata et al., 1972). As stated by Ou (1958), the deficiency might have decreased the resistance to RBLs resulting in higher disease incidences in organic and integrated systems compared to conventional. The susceptibility of RBLs development was found to be enhanced due to the formation of ammoniacal components, while it decreased with the formation of nitrates compounds (Chattopadhyay and Dickson, 1960; Imran et al., 2020). More often, infections of *B. oryzae*, occur through the epidermal cells instead of stomata (Nanda and Gangopadhyay, 1984) and the high leaf N facilitates more epidermal cells and higher thickness to impede fungal penetration. Further, high N affects plant resistance by reducing the frequency of successful penetrations by some pathogens or by slowing tissue colonization upon their penetration (Huber and Thompson, 2007) and by reducing the frequency of successful penetrations. Host resistance is also promoted by N associated with reduction of

Table 2. Cropping systems and their respective nutrient contents.

Cropping System	Mineral nutrient (kg.ha ⁻¹)	Nutrients from alternative sources (kg.ha ⁻¹)
CONV	N - 103.5 (Urea 46%)	N - 0
	P - 3.9 (P ₂ O ₅ 43.7%)	P - 0
	K - 30.0 (K ₂ O 60%)	K - 0
INT	N - 51.8 (Urea 46%)	N - 25.9
	P - 1.9 (P ₂ O ₅ 43.7%)	P - 0.65
	K - 15 (K ₂ O 60%)	K - 52.5
ORG	N - 0	N - 51.8
	P - 0	P - 1.3
	K - 0	K - 104.9

Table 3. Application of herbicides in CONV and INM during *Maha* 2018/2019, *Yala* 2019, *Maha* 2019/2020, and *Yala* 2020

Season	Herbicide		Time of application (days after sowing)	Rate of application (L.ha ⁻¹)	Dilution (product per 16L of water)
	Generic Name	Trade Name			
<i>Maha</i> 2018/19	Pretilachlor 30% EC	Sofit 30EC	0-4	1.6	64-80ml
	MCPA 60% SL	M60	18-21	1.8	72-89.6ml
<i>Yala</i> 2019	Pretilachlor 30% EC	Sofit 30EC	0-4	1.6	64-80ml
	MCPA 60% SL	M60	18-21	1.8	72-89.6ml
<i>Maha</i> 2019/20	Bispyribac sodium 4% + metamipof 10%	Kensolo	10-18 or weed at 2-5 leaf stage	0.1	4-5.12ml
<i>Yala</i> 2020	Pretilachlor 30% EC	Sofit 30EC	0-4	1.6	64-80ml
	MCPA 60% SL	M60	18-21	1.8	72-89.6ml

peptidase activity, lowering amino acids availability for pathogen nutrition, and inhibition of lytic enzymes involved in tissue maceration (Huber and Thompson, 2007).

Generally, modern rice cultivars like Bg300 are responding well to external application of N even with different sources of cropping systems, thus the organic systems were low yielding. The yields of conventional and integrated systems were more than two folds higher than the organic system (Table 4). The difference was more pronounced in the wet season 2018/19, however, was diminished with the continuous development of soil quality of both integrated and organic systems. During the latter part of the experiment, the yield was more stabilized, and the yield was more or less similar in the wet 2019/20 season onwards. During dry seasons, in terms of the yield, the integrated systems performed better compared to conventional and organic. In dry season 2020, three systems were similar, which might be attributed to multiple reasons (Table 5). A

study by Siavoshi and Laware (2013) also exhibited those organic materials have been found to cause a gradual improvement in soil productivity and crop performance over a long period. The similarity might have been attributed partly to the delayed disease progression and suppressed incidences of RBLs.

The rice yield and the RBLs disease incidences resulted in negative and significant correlations for all wet seasons and the dry season of 2019 (Table 5). The prevalence of RBLs had depressed the yield of the crop, while the impacts were severe when the crop is infected before anthesis. The ability of the rice crop to resist the disease by reducing the incidences was significantly changed with the source and the input level of N. Despite and early risk of higher incidences in an organic system, crops are grown under organic systems showed a better yielding ability with a greater ability to withstand RBLs. The uptake of organic acid from decaying organic residues might improve crop vigor in both integrated and organic systems (Ghorbani et al., 2009). Suppression of

Table 4. Effect of different cropping systems, growth stages and crop seasons on the yield, relative leaf chlorophyll content and leaf N concentration in wet and dry seasons

	Yield (ton.ha ⁻¹)		Relative leaf chlorophyll content (SPAD)		Leaf N (g.kg ⁻¹)	
	Wet	Dry	Wet	Dry	Wet	Dry
Cropping System (CS)						
CON	4.4 ^a		37.1 ^a		16.8 ^a	
INT	4.3 ^a		34.8 ^b		15.1 ^a	
ORG	3.2 ^b		29.1 ^c		12.0 ^b	
CV%	8.1		1.2		5.5	
Growth Stage (GS)						
PI	4.5 ^a	3.4 ^b	34.7 ^a	31.6 ^b	16.9 ^a	
Heading			34.5 ^a	33.9 ^a	12.4 ^b	
CV%	6.3		1.4		4.5	
Sources of Variance						
Cropping System (CS)	0.0199		<.0001		0.0001	
Growth Stage (GS)	NE		0.0236		<.0001	
Season (SE)	0.0036		0.0002		0.4434	
CS*GS	NE		0.7395		0.4476	
CS*SE	0.5509		0.2648		0.7758	
GS*SE	NE		0.0088		0.1628	
CS*GS*SE	NE		0.8842		0.4476	
Correlations between incidences at heading stage	-0.61 (<.0001)	-0.40 (0.03)	-0.38 (<.0001)	NA	-0.76 (<.0001)	NA

NE: not estimated; NA: not applicable; No incidences of disease were seen at this time. In the panicle initiation stage, the correlations with relative leaf chlorophyll content and leaf N were not calculated as there were no incidences of disease.

Table 5. Effect of cropping systems, growth stage and cropping season on the crop yield, relative leaf chlorophyll content and leaf N concentration and correlation between disease incidences and yield, relative leaf chlorophyll content and leaf N concentration in 2018/19, 2019/20 and 2020/21 wet seasons and 2019 and 2020 dry seasons.

Season	Cropping systems	Yield (ton ha ⁻¹)	Correlation between Incidence and Yield	Relative leaf chlorophyll content (SPAD)			Leaf N (g kg ⁻¹)			Date of Disease Incidence observation (DAS)
				PI	Heading	Correlation between Incidence and relative leaf chlorophyll content	PI	Heading	Correlation between Incidence and Leaf	
2018/19 Wet	CON	3.6 ^d	-0.41	38.8 ^{a-c}	35.9 ^{e-f}	-0.22	0.4 ⁿ	0.8 ^{kl}	-0.31	48
	INT	3.3 ^{de}	(0.019)	33.8 ^{f-i}	37.1 ^{a-d}	(0.21)	0.6 ^{mn}	0.8 ^{lm}	(0.07)	48
	ORG	1.6 ^f		28.2 ^{lm}	24.4 ⁿ		0.4 ⁿ	0.4 ⁿ		48
2019/20 Wet	CON	7.5 ^a	-0.69	36.3 ^{b-g}	36.3 ^{b-g}	-0.41	2.6 ^a	2.1 ^{c-e}	-0.34	60
	INT	5.6 ^b	(<.001)	35.6 ^{d-f}	34.6 ^{e-h}	(0.013)	2.6 ^a	1.8 ^{ef}	(0.05)	60
	ORG	5.7 ^b		29.7 ^{kl}	32.5 ^{h-j}		1.9 ^{de}	1.5 ^g		60
2020/21 Wet	CON	4.2 ^c	-0.33	39.2 ^a	39.2 ^a	-0.22	2.3 ^{bc}	1.9 ^{de}	-0.38	54
	INT	4.9 ^{bc}	(0.05)	37.2 ^{a-d}	36.7 ^{b-e}	(0.22)	2.1 ^{cd}	1.6 ^g	(0.08)	60
	ORG	4.1 ^c		33.9 ^{f-i}	34.3 ^{e-h}		2.0 ^{de}	1.2 ^{h-j}		54
2019 Dry	CON	4.9 ^{bc}	-0.61	36.3 ^{b-f}	37.1 ^{a-e}	NA	2.5 ^{ab}	1.4 ^{gh}	NA	87
	INT	5.1 ^{bc}	(0.012)	31.2 ^k	33.7 ^{f-i}		2.5 ^{c-e}	1.1 ^{h-j}		87
	ORG	3.0 ^{de}		25.8 ^{mn}	27.8 ^{lm}		1.4 ^{g-i}	0.9 ^l		81
2020 Dry	CON	2.2 ^{ef}	-0.09	34.7 ^{d-h}	38.8 ^{ab}	NA	1.6 ^g	1.2 ^{h-j}	NA	84
	INT	2.7 ^{def}	(0.77)	33.7 ^{f-i}	36.6 ^{a-f}		1.4 ^{gh}	1.1 ^k		84
	ORG	2.2 ^{ef}		27.9 ^{lm}	29.7 ^l		1.3 ^{g-i}	1.0 ^l		84
CV%		10.9		2.9	2.85		5.7	7.7		
Source of Variance										
Cropping System (CS)	**			**			**			
Growth Stage (GS)	NE			**			**			
Season (SE)	**			**			**			
CS*GS	NE			0.5548			0.7631			
CS*SE	**			**			**			
GS*SE	NE			**			**			
CS*GS*SE	NE			**			**			

NE: not estimated, NA: not applicable, No incidences of disease were seen at this time. In the panicle initiation stage, the correlations with relative leaf chlorophyll content and leaf N were not calculated as there were no incidences of disease.

Table 6. AUDPC values for the disease incidence of 2018/19, 2019/20 and 2020/21 wet seasons and 2019 and 2020 dry seasons under different cropping systems

Cropping systems	2018/19 Wet	2019/20 Wet	2020/21 Wet	2019 Dry	2020 Dry
CONV	2732.6	883.2	93.6	23.05	18.2
INT	2986.3	970.5	146.4	11	15.4
ORG	3082.9	1017.4	146.4	30.85	21

RBLS and probably other fungal diseases might have contributed to the yield stabilization in the latter seasons. Similar to the observation by Sunder et al., 2005, lower disease incidences in this study could have been associated with soil quality enhancement and changes in the crop vigour with long-term organic inputs. Furthermore, similar disease suppression has also been reported by Tajani et al. (1993) and Myint et al. (2007) in long-term organic studies. Therefore, the yield suppression due to RBLS disease in organic transition can be overcome by using an integrated approach and building a balanced substitutable nutrient management strategy in the paddy cultivation in the dry zone of Sri Lanka.

Conclusion

The organic input systems showed higher vulnerability to rice brown leaf spot disease, which was greatly attributed to the low N inputs. At the early stages of organic transition, disease prevalence was early, progression was quick, and incidences were greater in organic compared to conventional. Dryer seasons were more or less disease-free, or progressions were rather later despite N input or source. Long-term application of organic inputs can lead to resisting disease incidences and progression associated with the soil and related development of the system itself. The N management is essential to maintain correct ratios with either sole or a combination of inorganic and organic sources in the long run. In the provision of transitioning from a conventional system to organic, the yield suppression due to diseases such as rice brown leaf spot disease can be overcome by using an integrated approach and building a balanced substitutable nutrient management strategy.

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