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Full Length Research Paper

The nitrogen supply in wheat cultivation dependent on weather conditions and succession system in southern Brazil

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High wheat yields, besides the genetic potential and edaphoclimatic conditions, are obtained by proper management and nitrogen use. The objective of the study was to define the most appropriate time for N-fertilizer application, considering the range of greatest wheat requirements, dependent on the succession system type and the predictability of favorable and unfavorable years. The study was carried out in the 2008 to 2012 years, in Augusto Pestana, Rio Grande do Sul, Brazil. The experimental design was randomized blocks with four replications, with N-fertilizer application at 0, 10, 30 and 60 days after emergence, considering the corn/wheat and soybean/wheat succession system. The study found that the best time for nitrogen fertilizer application on wheat is mostly influenced by the year of cultivation and is less influenced by the succession system type. The appropriate time for the N-fertilizer application in favorable years of cultivation was about 45 days after emergence. In unfavorable years, it must be anticipated. Regardless of the cultivation year and the succession system type, the N-fertilization at 30 days after emergence evidenced the highest means as the most stabile grain yield.

Key words: Wheat *Triticum aestivum*, N-fertilizer time, optimization.

INTRODUCTION

In a globalized market, achieving self-sufficiency and competitiveness of Brazilian wheat is decisive. Therefore, the development of more productive cultivars, which are tolerant and efficient in the use of light and nutrients is

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desired (Freo et al., 2011). Besides the weather conditions, nitrogen management is essential to increase grain yield (Flores et al., 2012). The amount of fertilizer and the appropriate application time should also be taken into account due to the possibility that high doses and too early or too late applications might not be exploited at its most (Silva et al., 2005; Ma et al., 2010). In this context, the importance of nitrogen fertilization should be highlighted, not only for the costs associated to it, but also because it's efficient use is the key to sustainable production (Costa et al., 2013).

The N-fertilizer dose in wheat is based on the soil organic matter content, on the fore crop, and on the expected grain yield (Siqueira Neto et al., 2010). On the other hand, the appropriate time for nitrogen topdressing is defined by the plant phenology, according to the period of greatest deficiency of the nutrient during plant development and formation of yield components (Bredemeier et al., 2013). The periods when wheat mostly requires nitrogen is from the emergence until the sixth leaf stage (Yano et al., 2005). If applied in the early stages, the fertilizer promotes the maximum number of spikelet and grains per spike; if applied in final stages, it can increase the number of culms per area (Teixeira Filho et al., 2010). In Brazil, the technical recommendations for wheat production as the proper time of fertilization indicate the period between the beginning of tillering (V_3 stage; code 13 in BBCH scale) and the beginning of elongation (V_6 stage; code 16 in BBCH scale), that is, about 30 to 60 days after emergence stage, especially between the 30th and 45th day (Reunião da Comissão Brasileira de Pesquisa de Trigo e Triticale, 2013). It must be highlighted that the period between the beginning of tillering and elongation in wheat is indeed a large interval for the decision of the appropriate time of fertilizer application. This raises the need to consider factors other than just most favorable conditions of soil moisture, which would help in the definition of the more adjusted time of nitrogen supply, resulting in better efficiency in the nutrient use, and better grain yield.

The wide variation of grain yield is associated with the great variability in weather conditions, making the agricultural year the biggest contributing factor for the production instability (Storck et al., 2014). The years of favorable and unfavorable weather alter the nitrogen availability and its efficient use by the plant (Espindula et al., 2010). Also, it should be noted that nitrate leaching increases when N-fertilizer application is followed by excessive rain (Coelho et al., 2014), and ammonia volatilization is favored when applied in hot and dry periods (Ma et al., 2010). The type of vegetative cover also influences the losses by leaching or volatilization, and the nitrogen use efficiency (Ma et al., 2010; Viola et al., 2013). Therefore, the biochemical composition of residues affects the choice of proper dose and time of nitrogen supply, taking into account the nutrient release rate into the soil and the decomposing tissues (Siqueira Neto et al., 2010).

It is already known in the literature that weather conditions and cultivation techniques modify the nitrogen use efficiency. This justifies that the interactions between the succession system type and weather conditions and the periods of wheat greatest deficiency of nitrogen in wheat should be considered in the choice of the most appropriate period for application of N-fertilizer.

The objective of this study was to define the most appropriate period of N-fertilizer application according to wheat greatest requirement of the nutrient, dependent on the succession system type and on the predictability of weather favorable and unfavorable years.

MATERIALS AND METHODS

The experiments were carried out in the 2008 and 2012 years in the city of Augusto Pestana, RS, Brazil (lat. 28°26'30''S; long. 54°00'58''W, at 298 m asl). The soil is classified as Oxisol Distroferric Typical (Santos et al., 2006) and the climate, according to Köppen classification is Cfa with hot summer without dry season. The experiment was carried out in the same area during the five years, and it had had direct seeding for more than twenty years. The soil analysis carried out before sowings allowed to identify the following chemical characteristics: in corn/wheat succession system: Argil = 52%; Organic Matter = 2.9%; pH = 6.2; P = 40.8 mg dm^{-3}; K = 239.7 mg dm^{-3}; Al = 0.0 cmol$_c$ dm^{-3}; Ca = 6.5 cmol$_c$ dm^{-3} and Mg = 2.5 cmol$_c$ dm^{-3}; and in soybean/wheat rotation: Argil = 54%; Organic Matter = 3.2%; pH = 6.5; P = 26.9 mg dm^{-3}; K = 179.5 mg dm^{-3}; Al = 0.0 cmol$_c$ dm^{-3}; Ca = 6.3 cmol$_c$ dm^{-3} and Mg = 2.7 cmol$_c$ dm^{-3}. Sowings were carried out between May 15th and June 30^{th,} with a seeder-fertilizer. Each experimental field was formed of 5 m rows, spaced 0.20 m apart, composing the experimental unit of 5 m^2. Seeds underwent germination and vigor test in laboratory in order to correct the desired density of 330 viable seeds m^{-2} of the bread wheat cultivar BRS Guamirim, which has early cycle and short stature. During vegetation period, wheat plants were protected against diseases by tebuconazole fungicide applications of commercial name FOLICUR 200 EC® (Bayer CropScience Ltda, São Paulo, Brazil), at a dose of 0.75 l ha^{-1}. Moreover, weed control was carried out with metsulfuron-methyl herbicide, at a dose of 4 g ha^{-1}.

Wheat sowings basic fertilization (NPK) was applied in the rates of 80 kg ha^{-1} of P_2O_5, 60 kg ha^{-1} of K_2O and 10 kg ha^{-1} of nitrogen. The remaining part of nitrogen fertilization was applied in coverage in order to achieve an expected yield of 3 t ha^{-1} of wheat for each succession system. Therefore, 50 and 80 kg ha^{-1} nitrogen doses were applied in the soybean/wheat and in the corn/wheat succession systems, respectively. The experiments were set up in a randomized blocks experimental design with four replications, with N-fertilizer application (urea) at 0 (standard condition), 10, 30 and 60 days after emergence (DAE) of wheat plants. It should be noted that the periods of fertilization of 10, 30 and 60 DAE characterize the phenological stages of the wheat development V_1 (first expanded leaf), V_3 (third expanded leaf = early tillering) and V_6 (sixth expanded leaf = end of tillering and early elongation), respectively. Wheat was harvested at ripening (code 87 in BBCH scale). Grain yield was determined based in plants harvested manually from three central rows of each plot, which were then threshed with a stationary harvester. Grains were dried for humidity of 13% and weighed.

The obtained data on yield per hectare were subjected to analysis of variance for detection of interaction and for means

Table 1. Summary of the analysis of variance of times nitrogen application in wheat, in the different years and succession system.

Source of variation	DF	Mean square — Grain yield (kg ha^{-1})	
		Corn/wheat succession system	Soybean/wheat succession system
Block	3	43558	105077
Days after emergence	3	3374337*	2058141*
Year	4	3783418*	15555800*
Days after emergence x Year	12	433321*	112229*
Error	57	41745	57978
Total	79		
Overall mean		2236	2658
Coefficient of variation (%)		9.14	9.06

* = Significant at 5% confidence level by the F test; DF = Degrees of freedom.

grouping by the Scott & Knott model. Mean grain yield values along with information of temperature and pluviometric precipitation in the wheat vegetation period were used as criteria for classification of favorable and unfavorable years. Wheat yield stability was estimated according to Wrick (1965) and Eberhart and Russell (1966) models. The Wricke model, named ecovalence (ωi), was estimated according to the equation: $\omega_i = \sum_{j=1}^{n}(eN)_{ij}^2$, with the Nitrogen time $(eN)_{ij} = Y_{ij} - Y_i - Y_j - Y$, where Y_{ij} is the mean of time "i" in the environment "j"; Y_i is the mean of time "i" for all environments; Y_j is the mean of the environment "j" for all times; and $Y = m_i$ is the overall mean. According to this methodology, it is considered stable when reduced values of ωi or ωi (%) are observed. The Eberhart & Russell model is based on the linear regression: $Y_{ij} = B0_i + B1_i I_j + S^2_{ij} + E_{ij}$, where Y_{ij} is the mean time "i" in the environment "j"; $B0_i$ is the overall mean of the time "i"; $B1_i$ is the linear regression coefficient, whose estimate represents the response of the time "i" to the variation of the environment "j"; I_j is the codified environmental index; S^2_{ij} is the regression deviation; and E_{ij} is the experimental error expectation. The stability of the time of N-fertilizer application was obtained by the S^2_{ij} parameter. An indicator was considered stable when $S^2_{ij} = 0$, and unstable when $S^2_{ij} \neq 0$. Furthermore, regression equations were applied in order to define the study years (as favorable or unfavorable), and the most appropriate time of N-fertilizer application dependent on the succession system (soybean/wheat; corn/wheat). The statistical analyses were carried out with the aid of the GENES program (Cruz, 2006).

RESULTS AND DISCUSSION

Table 1 shows the analysis of variance, the interaction of N-fertilizer application time with the year of cultivation was detected, regardless the succession system. Therefore, the best time for the application was dependent on the year of cultivation, and the magnitude of the mean square shows major change in soybean/wheat succession system. The interaction between weather and the use of nitrogen in wheat results in variations from year to year in grain yield, being the availability of water the most decisive factor (Benin et al., 2012). The type of residuals coverage also affects the efficiency of fertilizer harnessing (Nascimento et al., 2012). In wheat, the use of leguminous plants has reduced the demand and the losses of nitrogen (Pinnow et al., 2013). The cultivation of wheat in succession to sunnhemp, the species of high rate of N-residual release, also promotes high yield, reduces the demand for fertilizers, and the losses of N by leaching and volatilization, and the total cost of the tillage (Nunes et al., 2011). Although the best use of fertilizer depends on the weather conditions and the succession system, genetic differences among cultivars also influence the rate of nitrogen uptake (Wamser and Mundstock, 2007). Besides the cultivars which have high grain yield those which are more efficient in fertilizer use, and are tolerant to environmental stresses have been continuously used (Oliveira et al., 2011).

Table 2 presents the corn/wheat succession system, the higher grain yield in the overall mean was obtained in the years of 2008 and 2011, which had values closer to the expected of 3 t ha^{-1}. Likewise, in the soybean/wheat succession system, 2008 and 2011 were the years with greater contribution to yield, surpassing the expectation of desired yield. It should be noted that in the years favorable for wheat cultivation in corn/wheat succession system, N-fertilizer applications were applied at 30 and 60 days after emergence (DAE). In the soybean/wheat succession system, this condition was also detected in 2008; however, the year of 2011 favored the application at 10 DAE along with the other times tested, except for the control treatment without fertilization.

The combined effect of years suitable for wheat cultivation (2008 and 2011) with the succession system of high N-residual release considerably contributed to increase grain yield. On the other hand, the unfavorable years nullified the benefits of soybean/wheat succession system (Table 2). Variations in temperature were not so high to the point of damaging the culture of wheat, except for particular conditions in 2009, in June and July, with minimum below 5°C (Table 3). Daily maximum

Table 2. Mean values of times of N-fertilizer application on wheat in the different years and succession system.

Days after emergence (DAE)	Corn/wheat succession system - Year				
	2008	2009	2010	2011	2012
0	A 1605^{c}	A 1604^{a}	A 1425^{b}	A 1717^{c}	A 1571^{b}
10	A 2671^{b}	C 1721^{a}	B 2015^{a}	A 2527^{b}	B 2134^{a}
30	A 3043^{a}	B 1919^{a}	B 2157^{a}	A 3204^{a}	B 2181^{a}
60	A 3268^{a}	B 1869^{a}	B 1815^{a}	A 3215^{a}	B 2042^{a}
Overall Mean	2647^{b+}	1779^{a+}	1853^{a+}	2666^{b+}	1982^{a+}
Days after emergence (DAE)	**Soybean/wheat succession system - Year**				
	2008	**2009**	**2010**	**2011**	**2012**
0	A 3160^{c}	C 1553^{b}	C 1301^{c}	B 2672^{b}	C 1672^{b}
10	A 3607^{b}	B 2050^{a}	C 1508^{c}	A 3389^{a}	B 2138^{a}
30	A 4079^{a}	C 2057^{a}	C 2257^{a}	B 3661^{a}	C 2213^{a}
60	A 4037^{a}	C 2105^{a}	D 1758^{b}	B 3334^{a}	C 2209^{a}
Overall Mean	3721^{a+}	1941^{a+}	1706^{a+}	3264^{a+}	2058^{a+}

Means followed by the same capital letter on the line, small letters in the column and with $^{+}$ sign represents the overall mean of each year between succession system, do not differ significantly by Scott & Knott test with 5% confidence level; DAE = days after emergence.

temperatures, even in the warmer months of the cycle, did not exceed the month average of 27°C. It stands out that, in the years which favored grain yield (Table 2), the total cumulative of pluviometric precipitation was similar to the average precipitation along the previous 25 years (Table 3). On the other hand, reduced grain yield was obtained in the years with precipitation excess (2009) and precipitation restriction (2010 and 2012), when compared to the long-term average. The analysis of the mean values of grain yield with the conditions of temperature and precipitation allowed to classify 2008 and 2011 as favorable years (FY) and 2009, 2010 and 2012 as unfavorable years (UY) to wheat cultivation (Table 3). The agronomic efficiency of nitrogen fertilization strongly depends on weather conditions (Battisti et al., 2013). Benin et al. (2012) observed that wheat was more responsive to grain yield increase by nitrogen fertilization when the precipitation was not a limiting factor. However, under favorable weather conditions, the use of high rates of nitrogen is not always the most appropriate strategy. The increase of grain yield can be achieved by improvements of the utilization efficiency of the fertilizer by the plant (Tavares et al., 2014).

The environmental variations, for being decisive in the time of fertilization, highlight the demand for high means with stability in wheat grain yield. This is a condition that qualifies the estimate of general means with the stability parameters in the five years of study at each fertilization time (Table 4). The use of N-fertilizer in the corn/wheat succession system indicates that the highest values of grain yield were obtained as a result of N applications at 30 and 60 DAE. At these development stages, the combination of high means with the lowest ecovalence value, and S^2_{ij} = 0 in the promotion of the greatest stability, was obtained with fertilization at 30 DAE. In the soybean/wheat succession system, the highest values of grain yield were also obtained with fertilization at 30 and 60 DAE. The greater N-residual condition enabled the high productivity with stability at the treatment of N fertilization at 60 DAE. This result confirms the effect of N-fertilizer application in cases under more restrictive conditions of N-residual release. On the other hand, the appropriate release of nitrogen contained in the tissues of soybean allows the possibility of nitrogen application delay under conditions of the soybean/wheat succession system. In the joint analysis involving succession systems (Table 4), the most stabile grain yield was correlated with fertilization at 30 DAE. Presented studies confirmed the previous results of Nascimento et al. (2012) and Benin et al. (2012), that plant residue decompose type affected the efficiency of nitrogen fertilization of

Table 3. Temperature and precipitation data in the months and years of wheat cultivation.

Year	Month	Temperature (ºC)			Precipitation (mm)		Class
		Minimum	Maximum	Mean	Mean 25 year*	Occurred	
2008	May	8.7	22.5	15.6	149.7	83.8	FY
	June	7.4	18.1	12.7	162.5	231.2	
	July	11.4	23.1	17.2	135.1	60.8	
	August	10.1	21.2	15.6	138.2	128.0	
	September	9.1	21.9	15.5	167.4	72.4	
	October	14.5	25.5	20.0	156.5	390.8	
	Total	-	-	-	909.4	967.0	
2009	May	10.7	24.4	17.5	149.7	189.0	UY
	June	4.6	17.9	11.2	162.5	55.6	
	July	4.3	17.5	10.9	135.1	137.2	
	August	10.1	23.0	16.5	138.2	268.2	
	September	9.8	21.9	15.8	167.4	348.0	
	October	11.7	26.7	19.2	156.5	126.9	
	Total	-	-	-	909.4	1124.9	
2010	May	8.0	13.8	10.9	149.7	149.7	UY
	June	8.5	14.5	11.5	162.5	119.8	
	July	7.7	16.9	12.3	135.1	225.3	
	August	7.2	18.1	12.6	138.2	41.7	
	September	8.4	24.9	16.6	167.4	145.1	
	October	11.8	25.6	18.7	156.5	109.4	
	Total	-	-	-	909.4	791.0	
2011	May	10.5	22.7	16.6	149.7	100.5	FY
	June	7.9	18.4	13.1	162.5	191.0	
	July	8.3	19.2	13.7	135.1	200.8	
	August	9.3	20.4	14.8	138.2	223.8	
	September	9.5	23.7	16.6	167.4	46.5	
	October	12.2	25.1	18.6	156.5	211.3	
	Total	-	-	-	909.4	973.9	
2012	May	11.1	24.5	17.8	149.7	20.3	UY
	June	9.3	19.7	14.5	162.5	59.4	
	July	7.4	17.5	12.4	135.1	176.6	
	August	12.9	23.4	18.1	138.2	61.4	
	September	12.0	23.0	17.5	167.4	194.6	
	October	15.0	25.5	20.2	156.5	286.6	
	Total	-	-	-	909.4	798,9	

* = Mean of pluviometric precipitation obtained based on the months from May to October 1982-2007; Class = classification suggested by the authors; FY = favorable year; UY = unfavorable year.

the culture in succession. Furthermore, growing wheat after soybeans, when compared to corn/wheat succession system, greatly increases grain yield with the same fertilization due to the greater availability of N-residual (Bredemeier et al., 2013).

The predictions of the optimal time of N-fertilizer application for the conditions of favorable and unfavorable year of cultivation, and the regression equations that indicate the need for early or late applications are presented in Table 5. It is noteworthy that in the different succession systems, only favorable years (2008 and 2011) showed significant linear trend for

Table 4. Stability parameters on grain yield dependent on the application time of N-fertilizer in succession system by the Wricke model and Eberhart & Russell model.

Days after emergence (DAE)	Mean of grain yield ($kg\ ha^{-1}$)	Ecovalence ωi	Ecovalence ωi (%)	Regression S^2_{ij}	Regression R^2 (%)
		Corn/wheat succession system			
0	1587^c	2075079	53.3	1767^{ns}	44.2
10	2233^b	197904	5.0	9170^{ns}	93.3
30	2580^a	333655	8.5	-773^{ns}	98.4
60	2541^a	1293254	33.2	-718^{ns}	99.0
		Soybean/wheat crop roation			
0	2171^c	132556	13.2	-12538^{ns}	99.8
10	2638^b	312704	30.9	23843^{ns}	97.5
30	3013^a	389679	38.6	34180*	96.8
60	2808^a	175121	17.3	-283^{ns}	99.2
		Joint analysis (corn/wheat + soybean/wheat)			
0	1879^c	2417195	45.5	64592*	84.9
10	2436^b	513000	9.7	8590^{ns}	96.8
30	2796^a	724178	13.6	15105^{ns}	96.4
60	2675^a	1660146	31.2	43993*	93.7

Means followed by the same letter in the column do not differ at 5% confidence level (by the Scott & Knott test); DAE= days after emergence; Ecovalence (ωi) = Wricke; Regression = Eberhart & Russell; * significant at 5% confidence level by the F test; ^{ns} not significant by F test; S^2_{ij} = deviations from regression; R^2 = coefficient of determination by the *F* test (Ho: S^2_{ij} = 0).

grain yield. Under corn/wheat rotation, in the favorable years (2008 and 2011), the best time for fertilization in coverage was at 44 and 45 DAE. In the years considered as unfavorable (2009, 2010 and 2012), the demand for anticipation of the time of fertilization is evident, with the best weather condition around 34 and 35 DAE, that is, an anticipation of 10 to 11 days (Table 5). In the soybean/wheat succession system (Table 5) this trend was also observed, showing the best time of fertilization in coverage at 48 DAE in favorable years, and around 39 and 40 DAE, in unfavorable years, that is, an anticipation of 8 to 9 days. The succession system presented less correlation to the ideal time of fertilization (3 to 5 days) compared to the effect of the year of cultivation. These results demonstrate that the year of cultivation is decisive for better nitrogen use, considering the ideal time of fertilization for grain yield. The use of residual N in the soybean/wheat succession system provided considerable benefits to the grain yield of wheat. The additive effects of weather conditions in a favorable year in this succession system and the best time of nitrogen supply enables grain yield superior to 4 $t\ ha^{-1}$ (Table 5).

As previously reported, the favorable and unfavorable conditions in the year of cultivation were predominantly defined by the pluviometric precipitation (Figure 1). The 2008 and 2011 years, considered as favorable, evidenced the occurrence of precipitation close to the nitrogen application time, with the exception of 2011, when the fertilizer was applied at 10 DAE. On the other hand, in the years 2009, 2010 and 2012, most of the times there was no occurrence of rainfall close to the nitrogen application time (Figure 1). Therefore, the nitrogen use efficiency is reinforced when the fertilizer was applied in the interval between 30 and 60 DAE, in periods near the occurrence of precipitation, regardless the succession system, as long as precipitation is not of high pluviometric volume. These results emphasize the importance of understanding the effect of year climatic conditions and management practices in wheat productivity. Stresses caused by deficiency or excess of water in the soil negatively affect the plant development, with direct effect on ultimate yield (Guarienti et al., 2005). The most efficient way to reduce these risks is the choice of proper cultivar, the sowing time (Silva et al., 2011), and the dose and time of nitrogen application.

Conclusions

The choice of the best time for nitrogen dressing on wheat was strongly influenced by the weather conditions distribution on the year of cultivation; however, it is less influenced by the succession system.

In favorable years for wheat cultivation, the best time for fertilization with N-fertilizer was around 45 days after emergence. In unfavorable years, the N-fertilizer should

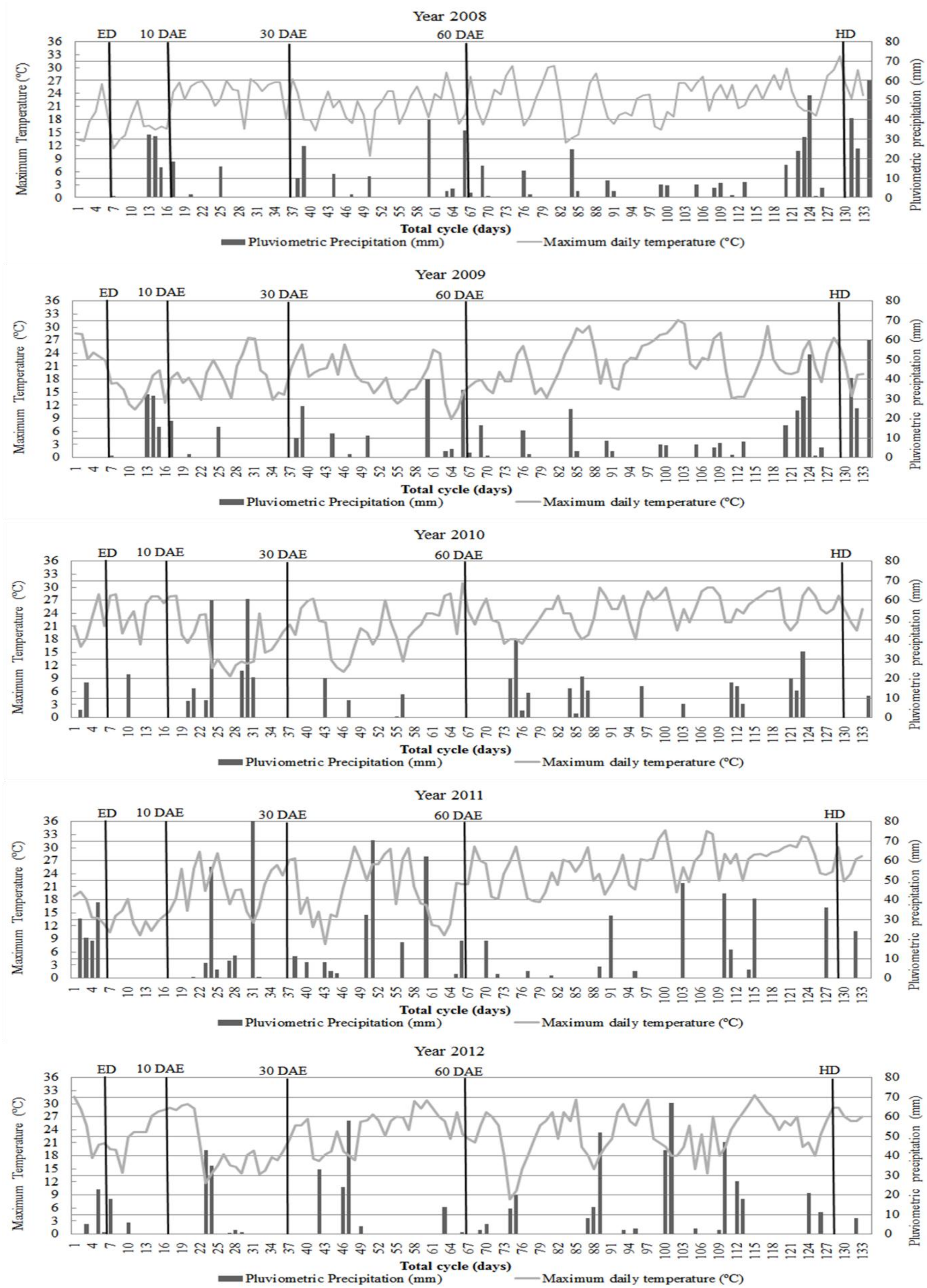

Figure 1. Climatological data of different years of study; ED = Emergence day; DAE = Days after emergence; HD = Harvest Day.

Table 5. Regression equations and the ideal time estimate of nitrogen supply for wheat grain yield in the different years and succession system.

Year	Degree	Regression Model $GY = a \pm bx \pm cx^2$	R^2	P	The best time (DAE)	GY_E (kg ha^{-1})
		Corn/wheat succession system				
2008-FY	1	2058 + 23.52x	0.71	*	45	3383
	2	1758 + 71.22x -0.78x^2	0.92	*		
2009-UY	1	1670 + 4.33 x	0.64	ns	35	1882
	2	1594 + 16.28x - 0.23x^2	0.99	*		
2010-UY	1	1752 + 4.02x	0.61	ns	34	2242
	2	1502 + 43.86x - 0.65 x^2	0.90	*		
2011-FY	1	2097 + 22.72x	0.72	*	44	3432
	2	1768 + 75.23x - 0.85x^2	0.99	*		
2012-UY	1	1894 + 4.65x	0.78	ns	34	2150
	2	1656 + 29.14x - 0.43x^2	0.89	*		
		Soybean/wheat succession system				
2008-FY	1	3380 + 13.62x	0.70	*	48	4193
	2	3071 + 46.89x - 0.49x^2	0.99	*		
2009-UY	1	1769 + 6.86x	0.69	ns	40	2167
	2	1648 + 26.16x - 0.33x^2	0.77	*		
2010-UY	1	1495 + 8.41x	0.60	ns	39	2156
	2	1108 + 54.20x - 0.70x^2	0.91	*		
2011-FY	1	3057 + 18.28x	0.97	*	48	4092
	2	2754 + 56.52x - 0.59x^2	0.93	*		
2012-UY	1	2425 + 9.29x	0.69	ns	39	2042
	2	1163 + 45.95x 0.60x^2	0.91	*		

FY = Favorable year; UY = Unfavorable year; * significant at 5% confidence level by the *t* test; ^{ns} not significant by *t* test; Degree 1 and 2 = linear and quadratic regression, respectively; R^2 = coefficient of determination, in decimal; P = significance level; significant (*), or not significant (^{ns}); GY_E = Estimated grain yield from the best time in kg ha^{-1}.

be applied about 35 days after emergence. Regardless of the year of cultivation and succession system, N-fertilization at 30 days after emergence enables high stability of grain yield.

Conflict of Interests

The authors have not declared any conflict of interests.

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Oxisolphysical attributes under different agricultural uses in Brazil

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Soil attributes are easily modified for different agricultural purposes, requiring the adoption of appropriate practices, according to the local particularities in order to maintain its production capacity. Given the above, this study aimed to assess soil physical attributes under different agricultural uses conditions in an Oxisol. The survey was conducted in Brazil with the following treatments: Cassava monoculture area, beans monoculture and native forest. Results were interpreted with multivariate analysis. Native forest differed from the other agricultural uses due to surface consolidation, lack of soil preparation practices and increased organic matter input, having higher water retention. The conclusion was that there were statistical irregularities in soil attribute tendencies. Different agricultural uses interfered in the physical attributes when compared to the native forest, which produced better results, followed by bean and cassava monocultures. The most significant physical attributes to distinguish agricultural uses were: Aggregation; weighted average diameter; soil density; particle density; total porosity; field capacity and available water.

Key words: Soil properties, density, porosity, water retention.

INTRODUCTION

Soils are three-dimensional, natural and dynamic bodies. They are formed on the Earth's surface through climate and organisms environmental factors action on the source material in function of relief, time action and

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spatial variation, according to these factors combination possibilities of these factors (Kämpf and Curi, 2012).

Under natural conditions the soil is in dynamic equilibrium and is resistant to changes (Goedert and Oliveira, 2007). However, human action has promotes attribute changes (Silva et al., 2007), causing soil natural fertility decline (Cordeiro et al., 2004), as its structure is easily changed. Thus, other properties are also degraded, such as soil density, micro, macro and total porosity, organic matter, water retention and infiltration in the soil profile (Reichert et al., 2009; Ferreira et al., 2010; Cunha et al., 2011).

Therefore, inappropriate land use causes changes in physical and hydraulic properties. The magnitude of these changes magnitude varies depending on the soil type, weather and agricultural crops management (Kay, 1990).

In general, arid and semiarid regions soils are naturally fragile because of their low water storage. In addition, plant root system growth is also physically limited in these soils (Cardoso, 2002), increasing its susceptibility to human action improper use practices.

Soil studies in Rio Grande do Norte state western region and in the municipality of Martins, RN state, are scarce. Therefore, physical attributes quantification is necessary to establish appropriate farming practices establishment, in order to seek better conditions in relation to the soil water dynamics, aeration and structure, which are dynamic attributes with spatial and temporal variability.

Given the above, this study aimed to assess soil physical properties under different agricultural uses in Martins, RN. Multivariate analysis was used as a tool to interpret results.

MATERIALS AND METHODS

Research was conducted in Martins, RN, Brazil. Martins is located in the Rio Grande do Norte state western meso region and in the Umarizalmicro region, in the following geographical coordinates: 6° 05´ 16´´ South, 37° 54´ 40´´ West. It is located in the Borborema plateau. However, its relief further comprises the Depressão Sertaneja, covering an area of 169.47 km². Climate is classified as tropical rainy (Aw) according to Köppen classification, with average rainfall of 1133.8 mm, whose rainy season is from January to June, the average annual temperature is of 25°C and subperennial forest vegetation type, which is associated with the Caatinga vegetation.

The following treatments were assessed: cassava monoculture (CASS), bean monoculture (BEAN) and native forest (NF).

The cassava monoculture area covered about 1 ha. The site was cleared in 2003. Plant residues were burned in small piles, with plowing and harrowing being conducted afterwards. These practices were always adopted before cassava planting, about once every two years. Sampling was performed after burning.

The bean monoculture area covered 1 ha, and like the cassava area, it was deforested in the same year (2003). Plant residues were burned and soil tillage was held afterwards through plowing and harrowing, the latter being held once a year, before sowing.

Through visual analysis by the farmer, cattle manure was applied to improve soil fertility. However, such practice was not performed during sampling.

The native forest area was used as a reference to compare with the other agricultural uses. The vegetation is of subperennial type, consisting of broad-leaved trees, with relatively slender and dense trunks, which is typical of tropical rainy climate zones. The most commonly found species are: *Psiium firmum*, *Talisia esculenta*, *Enterolobium contortisiliquum*, *Pithe colobium* polycephalum, *Sideroxylon obtusifolium*, Helicostylistomenosa, *Hymenaea courbaril*, *Dipteryx odorata*, *Mimosa sepiaria*, *Copaifera langsdorffii,* among others.

Agricultural uses textural classification was: sandy clay to native forest (NF), cassava monoculture (CASS) and bean monoculture (BEAN). Two soil profiles were opened in a representative area of the study sites. Soil samples with deformed structure in their respective horizons were collected for physical analysis, as well as diagnostic horizons identification and classification, according to the Brazilian Soil Classification System (Donagema et al., 2011; Santos et al., 2013).

Profile 1 showed a textural classification ranging from sandy clay on A and BA horizonsto clay in the Bw horizon. Profile 2 ranged between sandy clay in the A horizon to clay in the Bw horizon. Both were classified as Oxisol.

In order to assess soil physical properties, four representative points in each area were sampled to collect disturbed and undisturbed samples, in depths from 0.00 to 0.30 m. Subsequently, two representative profiles of the areas under study were opened for soil classification of soil, according to methodology described by the manual of methods and soil analysis (Donagema et al., 2011).

In order to collect soil samples with undisturbed structure an Uhland type device was used, with cylindricalmetal sampler dimensions of 0.05 m diameter and 0.05 m height was used. Physical attributes, retention curve, soil density, total porosity, macro and microporosity were assessed. Eight samples were collected by point and 32 samples were collected for each area under study, totaling 128 samples. After collection, samplers were coated with aluminum foil and taken to the laboratory with care, in order to maintain the soil structure and moisture.

Deformed structure samples collection were carried out in four representative points of each area, with three repetitions, totaling 48 samples, Collection was conducted with the aid of a straight shovel, with samples being transferred to properly labeled plastic bags. Subsequently, samples were used to determine textural classification and particle density.

Textural classification (granulometric analysis) carried out by pipette method using chemical dispersant (sodium hexametaphosphate). Soil density (SD) was determined by volumetric ring method and particle density (PD) was determined by volumetric flask method, using alcohol. These determinations were performed following Embrapa soil analysis methods manual (Donagema et al., 2011).

In order to determine aggregation and average diameter, wet screening method was used. Five samples of 25 g for each agricultural use were used, with a sample being used for moisture determination (correction factor). Each sample was transferred to a filter paper disk located on the upper sieve (2.00 mm) and spread over the entire filter paper surface. Moistening was conducted through capillarity (4 min) in a four sieves set with the following mesh diameters: 2.00; 1.00; 0.50 and 0.25 mm.After the required time, the filter paper was removed. Sieve sets agitation was performed in avertical oscillation apparatus (42 oscillations/min) during four minutes inside barrels with water. Fractions retained on each sieve were transferred to beckers that were previously treated and identified, and sent to an oven at 105°C for 24 h. After dry

Table 1. Physical attributes analysis of variance (ANOVA) under different agricultural uses: cassava monoculture (CASS), bean monoculture (BEAN) and native forest (NF).

VS	DF	MS									
		AGREG	WAD	SD	PD	TP	MICRO	MACRO	FC	PWP	AW
Between AU	2	6.92^{ns}	0.02**	0.01^{ns}	0.001^{ns}	91.58*	43.00^{ns}	47.58^{ns}	0.01^{ns}	0.001*	0.005^{ns}
WithinAu	9	1.70	0.001	0.01	0.01	12.39	15.81	13.83	0.002	0.0001	0.002
CV (%)		14.04	13.30	6.70	2.94	8.45	30.01	13.20	19.18	7.64	36.85

VS – variation source; AU - agricultural uses; CV - coefficient of variation; DF - degrees of freedom; MS - mean square; AGREG. - Aggregation; WAD – weighted average diameter; SD - soil density; PD - particle density; TP; total porosity; MICRO - microposity; MACRO - macroporosity; FC - field capacity; PWP – permanent wilting point; AW - available water; ** Significant difference indicator of p≤0.01; * Significant difference indicator of p≤0.05; ns - not significant.

matter obtainment and sand content (sodium hexametaphosphate) deduction, water stable aggregates (aggregation) percentage and weighted average diameter (WAD) were determined for each class. WAD was obtained through Equation 1, proposed by Castro Filho et al. (1998).

$$\mathrm{WAD} = \sum_{i=1}^{n} (xi\ wi) \quad (1)$$

Where: WAD–weighted average diameter, mm; xi–classes average diameter, mm; wi - each class proportion in relation to the total.

For TP, macroporosity and microporosity determination, tension table was used. For microporosity, a tension of 6 kPa was used. Macroporosity was calculated through the difference between total porosity and microporosity.

The graphical relation between matric potential and soil water content is calledsoil water characteristic curve, or moisture retention curve, that is, matric potential (φm) x water content (θ). For retention curve obtainment, tensions of 0; 2; 6; 10; 33; 100; 300; 500 and 1500 kPa were used. In the application of 0; 2; 6 and 10 kPa tensions, the tension table was used. Low tension cameras were used for 33 and 100 kPa, and high tension cameras were used for 300; 500 and 1500 kPa.

Soil water retention curves adjustment was made based on van Genuchten's equation (1980), (Equation 2) using the SWRC software, developed by Dourado Neto et al. (1990). The equation considers the matric potential (φm) as an independent variable, and water content (θ) as dependent variable:

$$\theta = \theta_r + \frac{\theta_s - \theta_r}{\left[1 + \left(\alpha.|\varphi_m|\right)^n\right]^m} \quad (2)$$

Where: θr -residual water content, $m^3\ m^{-3}$; θs- saturated water content, $m^3\ m^{-3}$; |φm|- matric potential, kPa α, m, n –Equation empirical parameters.

As it is a theoretical parameter that varies between 10 kPa (sandy soils) and 33 kPa (clay soils), field capacity value (FC) was determined according to the textural classification. Permanent wilting point (PWP) was obtained with 1500 kPa tension, and available water (AW) was obtained through the difference between FC (at 10 kPa tension) and PWP.

Soil physical attributes average values under different agricultural uses (NF, CASS and BEAN) were interpreted through ANOVA, which was performed with Tukey's test (p<5%), descriptive analysis (average, minimum, maximum, variance, standard deviation and coefficient of variation) In addition, multivariate statistical analysis tool was used in Principal Component Analysis (PCA), using the GENES program.

Soil physical attributes were measured in different unit systems, with data standardization being necessary, since variance is influenced by the attributes in the measurement units in question. Correlation matrix was established after data standardization, in order to verify the percentage and the degree of importance of these correlations, with values that were higher or equal to 0.7 being considered.

RESULTS AND DISCUSSION

Through soil physical attributes analysis of variance under different agricultural uses (AU analysis of variance, it was shown that there was no statistical difference for AGREG, SD, PD, MACRO, MICRO, FC and AW there was no statistical difference. WAD statistically differed at1% probability and TP and PWP were statistically different (p<0.05) (Table 1). These differences are probably due to soil structural arrangement being a dynamic, functional, functional and complex property that is easily modified property by land use (Carneiro et al., 2009; Kämpf and Curi, 2012).

Evaluating SD, PD, TP and PWP attributes coefficients of variation (CV) showed low values (CV lower than 10%); as for aggregation, WAD, MACRO and average FC, CV was between 10 and 20%. For MICRO and AW, CV was very high (higher than 30%), according to Pimentel (2009).

The correlation matrix obtained with the physical attributes studied in the different agricultural uses (NF, CASS, BEAN), in the municipality of Martins-RN were highly correlated (values above 0.7), showing interrelation in the different agricultural uses (Table 2). High and positive correlations between aggregation (AGREG), weighted average diameter (WAD) and microporosity (MICRO) were found.

High positive correlations were found between SD and TP for MACRO, FC and AW (except for TP, in which high

Table 2. Soil physical attributes correlation matrix obtained by principal component analysis (PCA) under different agricultural uses (CASS, BEAN and NF).

	AGREG.	WAD	SD	PD	TP	MICRO	MACRO	FC	PWP	AW
AGREG.	1.00									
WAP	0.99	1.00								
SD	-0.06	0.08	1.00							
PD	-0.88	-0.79	0.42	1.00						
TP	0.09	0.22	0.99	0.29	1.00					
MICRO	0.77	0.85	0.59	-0.45	0.70	1.00				
MACRO	-0.67	-0.57	0.78	0.86	0.68	-0.05	1.00			
FC	0.13	0.29	0.99	0.29	1.00	0.74	0.64	1.00		
PWP	0.64	0.77	0.59	-0.22	0.69	0.90	0.04	0.74	1.00	
AW	-0.10	0.05	1.00	0.47	0.98	0.56	0.80	0.96	0.63	1.00

AGREG. - Aggregation; WAD – weighted average diameter; SD - soil density; PD - particle density; TP; total porosity; MICRO - microposity; MACRO - macroporosity; FC - field capacity; PWP – permanent wilting point; AW - available water.

positive correlation was found for MICRO). These correlations can be justified due to SD and TP not being limiting factors for other attributes, with soil density values ranging from 1.14 to 1.28 g cm^{-3} and total porosity ranging between 37 and 47% (Table 3). According to Borges et al. (1997), SD values above 1.62 g cm^{-3} were partial obstacles for root growth while working in an Oxisol with medium texture at the *Triângulo Mineiro* region.

In the native forest, higher AGREG, WAD, SD, TP, MACRO, FC and AW average values were found (Table 3), what can be attributed to its surface higher consolidation due to tillage practices absence and higher organic matter input provided higher average values. Data tendency is in accordance with Corrêa (2002), who found increased aggregates and weighted average diameter innative forest, with reduction as intensive land use practices were carried out. Different agricultural management practices interfered in aggregates formation because it modifies soil organic matter dynamics (Zanatta et al., 2007) and conditions for microorganism activity (Vargas and Scholles, 2000).

Considering the principal component 1 (PC1), which showed 55.26% of total data variation, physical attributes that had the highest weights (in module) were: soil density (SD), total porosity (TP), field capacity (FC) and available water (AW). In CP2, which explained 41.46% of data explanation, the largest eigenvectors were: Aggregation (AGREG.), weighted average diameter (WAD) and particle density (PD) (Table 4).

In the graphic representation (Figure 1), the two first principal components scores (PC1 and PC2) were considered for groups' interpretation. Two different groups were formed; the first refers to cassava (CASS) and beans (BEAN) monoculture, indicating that practices adopted in these areas have not contributed to physical attributes differentiation. Analyzing the second group, native forest (NF), it differed from the first group (CASS and BEAN), being higher regarding soil physical properties. Albuquerque et al. (2005), Souza et al. (2005), Carneiro et al. (2009), Corrêa et al. (2010) obtained similar results, as agricultural use has changed soil physical properties, when compared to the native vegetation area.

Assessing soil water retention curve under different agricultural uses (NF, CASS and BEAN), which was adjusted according to the mathematical model proposed by Van Genuchten (1980), (Figure 2) the different agricultural uses showed uniformity, except for cassava monoculture, at 0 to 100 kPa tensions, which were below the others, due to lower total porosity (Table 3).

Water retention process occurs in unsaturated soils as a result of capillarity. Adsorption forces operating in the soil matrix vary depending on the texture, mineralogy, depth, structure, porosity, organic matter and agricultural uses (Libardi, 2005; Reichardt and Timm, 2004).

Viscosity is a property that reflects the ease with which the molecules or particles slide over the other, being directly proportional to the volume of the particles varying with the temperature. It is affected by solutes type and concentration of solutes (Reichardt and Timm, 2004).

Native forest (NF) showed higher water retention, total porosity, field capacity and available water. This can be justified by OM increase due to vegetation cover maintenance, soil non-disturbance and iron aluminumoxides presence, which give higher aggregate stability. Results obtained by (Roth et al., 1991; Bertol et al., 2000; Giarola et al., 2002) corroborate with this study. Soil and agricultural crops management modify the soil structure, and consequently, its physical properties, such

Table 3. Physical attributes descriptive statistics under different agricultural uses: native forest (NF), cassava monoculture (CASS) and bean monoculture (BEAN).

Physical attributes	Management	Descriptive analysis					
		Md	Min	Max	s^2	s	CV
AGREG. (%)	NF	10.00	8.89	11.18	0.90	0.95	9.48
	CASS	10.09	7.38	11.44	3.38	1.84	18.2
	BEAN	7.77	6.68	8.62	0.82	0.91	11.7
WAD (mm)	NF	0.26	0.23	0.29	0.00	0.03	10.4
	CASS	0.24	0.21	0.29	0.00	0.04	14.8
	BEAN	0.15	0.12	0.17	0.00	0.02	14.4
SD (g cm^{-3})	NF	1.28	1.25	1.31	0.00	0.02	1.91
	CASS	1.16	1.09	1.3	0.01	0.10	8.36
	BEAN	1.22	1.11	1.35	0.01	0.10	8.22
PD (g cm^{-3})	NF	2.53	2.39	2.61	0.01	0.10	3.80
	CASS	2.52	2.43	2.60	0.00	0.07	2.78
	BEAN	2.55	2.48	2.59	0.00	0.05	1.96
TP (%)	NF	47.00	0.45	0.49	0.00	0.02	3.65
	CASS	37.00	0.33	0.42	0.00	0.04	10.8
	BEAN	41.00	0.35	0.45	0.00	0.04	10.4
MICRO (%)	NF	17.00	0.16	0.19	0.00	0.02	8.96
	CASS	13.0	0.09	0.22	0.00	0.06	48.9
	BEAN	10.00	0.07	0.13	0.00	0.03	24.4
MACRO (%)	NF	30.00	0.28	0.3	0.00	0.01	3.39
	CASS	24.00	0.20	0.28	0.00	0.03	13.6
	BEAN	31.00	0.25	0.38	0.00	0.05	17.7
FC (cm^3 cm^{-3})	NF	0.29	0.26	0.31	0.00	0.02	8.45
	CASS	0.21	0.16	0.27	0.00	0.05	26.6
	BEAN	0.24	0.17	0.28	0.00	0.05	23.2
PWP (cm^3 cm^{-3})	NF	0.14	0.13	0.14	0.00	0.01	3.64
	CASS	0.12	0.11	0.13	0.00	0.01	6.80
	BEAN	0.11	0.10	0.13	0.00	0.01	11.20
AW (cm^3 cm^{-3})	NF	0.15	0.12	0.18	0.00	0.03	18.10
	CASS	0.09	0.03	0.15	0.00	0.06	64.80
	BEAN	0.13	0.07	0.17	0.00	0.05	37.30

AGREG. - Aggregation; WAD – weighted average diameter; SD - soil density; PD - particle density; TP; total porosity; MICRO - microposity; MACRO - macroporosity; FC - field capacity; PWP – permanent wilting point; AW - available water; NF - native forest; CASS – cassava monoculture; BEAN - bean monoculture; Md - mean; Min - minimum; Max - maximum; s^2 - variance; s - standard deviation; CV - coefficient of variation.

as density, porosity (Faria et al., 1998; Pires et al., 2012) and soil water retention (Ramos et al., 2013). Therefore, management based on monoculture, compared to native forest, negatively influenced on physical and hydraulic attributes (Table 3 and Figure 2). In Brazil Portela et al. (2001), among others, Cintra et al. (2009), found modifications in soil pores quantity and quality. The soils of both studies were classified as Oxisol in water retention, in a tableland ecosystem soil under different land use.

Conclusions

Different agricultural uses influence soil physical attributes mean values. The most significant physical

Table 4. Soil physical attributes eigenvectors analyzed with the main components.

Soil physical properties	Principal components	
	1	2
AGREG.	0.09	0.48
WAD	0.15	0.46
SD	0.41	-0.13
PD	0.09	-0.46
TP	0.42	-0.06
MICRO	0.33	0.30
MACRO	0.24	-0.40
FC	0.42	-0.04
PWP	0.34	0.24
AW	0.40	-0.15
Eigenvalue	5.29	4.20
Explanation (%)	55.26	41.46
Accumulated explanation (%)	41.46	96.72

AGREG. - Aggregation; WAD – weighted average diameter; SD - soil density; PD - particle density; TP; total porosity; MICRO - microposity; MACRO - macroporosity; FC - field capacity; PWP – permanent wilting point; AW - available water.

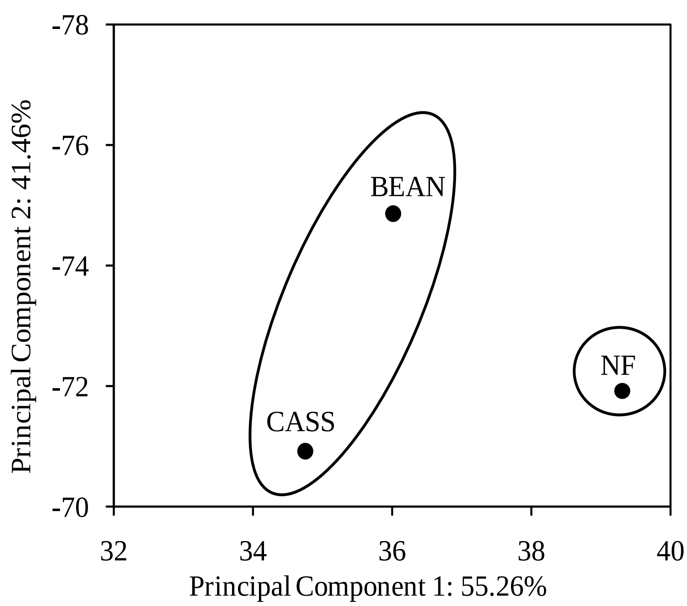

Figure 1. Scores graphical representation in the different agricultural uses: cassava monoculture (CASS), bean monoculture (BEAN) and native forest (NF), obtained with soil physical attributes.

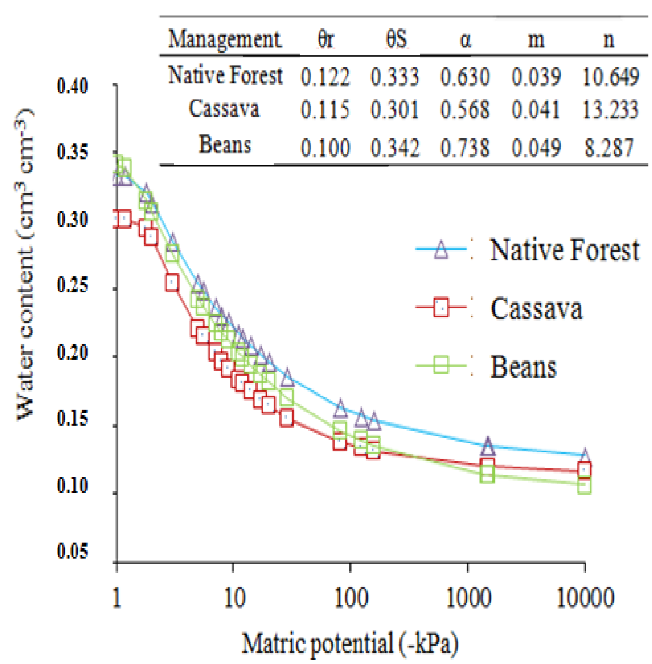

Figure 2. Soil water retention curve of an Oxisol under different agricultural uses: cassava monoculture (CASS), bean monoculture (BEAN) and native forest (NF).

attributes to distinguish agricultural uses were: aggregation; weighted average diameter; soil density; particle density; total porosity; field capacity and available water. Native forest obtained better results, followed by cassava and beans monoculture. Soil water characteristic curves showed uniformity among different agricultural uses with higher water retention in the native forest.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

The distribution of plant-parasitic nematodes of *Musa* spp. in Nsukka Agricultural Ecological zone, Enugu State, Nigeria

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Studies on the root nematodes of *Musa* spp. in Nsukka Agro-ecological zone of Enugu state, Nigeria, was carried out in sixteen towns located in this zone. The study involved collecting data on the distribution, incidence and abundance of nematode parasites on *Musa* spp., generating information on geo-physical, agricultural and historical data in the study area, examining nematode egg mass burden in *Musa* spp. roots and determining the physico-chemical properties of the soil as well as evaluating thier relationships with nematode density. This was obtained through questionnaire admistration to the farmers and root sample collection from sixteen towns in the study area. The collection of the root samples were done from tree villages in each town, three sites in each village and three sampling units in each site giving rise to 144 units each for *Musa* spp. (Banana and plantain). Both cluster and statified random sampling methods were used to collect root samples. The soil samples were used for physico-chemical analyses and nematode extraction. The Baerman's funnel method was used to estimate the nematode population in the root and soil samples. Data generated were subjected to descriptive statistics involving two way analysis of variance, calculation of means and standard deviation, the least significant differences at 5% probability level. Regression analysis was performed with the aid of the Statistical Package for Social Sciences (SPSS). Results of field survey showed that on the average, 67.51% of *Musa* spp. were grown in homestead while 24.65% were grown in farmlands, with 7.87% grown in the wild. Nematode parasites were evenly distributed with Meloidogyne spp and Radopholus similis as the most dominant and diverse species. Integrated management concept among other recommendations were proffered to reduce or probably eliminate the spread of nematode parasite and inproved yield of the *Musa* spp. plant.

Key words: Root nematodes, distribution, physico-chemical properties, *Musa* spp., Nsukka Agro-ecological zone.

INTRODUCTION

Nematodes are worms of the large phylum nematode such as round worm or thread worm. They are the most numerous multicellular animals on earth (De Waele and Elsen, 2007). A handful of soil will contain thousands of

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the microscopic worms, many of them parasites of insects, plants or animals. Bridge (2000) found out that there are 17 species of *Musa* root nematodes in Africa that are recognized to have possible economic importance of which most attention was directed at *Radopholus similis, Pratylenchus goodeyi, Pratylenchus coffeae, Helicotylenchus multicinctus, Hoploaimus pararobustus and Meloidogyne* spp.

The root-knot group of nematodes is the most serious plant parasitic nematodes because of their adaptability, pathogenicity, worldwide distribution in temperate and tropical climates and extensive host range that includes most economic plants (Filipjev and Schuurmans, 1941).

Banana weevil *Cosmopolites sordidus* (Germar), *Coleoptera curculionidae*) and parasitic nematodes are often found together on the same banana mat (Hill and Waller, 1999). Musabyimana et al. (2000) in Kenya have recognized both pests as major constraints to banana production, causing up to 85% yield loss in banana. However, Nigerian Agriculture is under the burden of destructive root nematodes that is rather known as plant parasitic nematode (Agrios, 2005). Adediran et al. (2005) conducted a study in three agro- ecological zones of South Western Nigerian to evaluate the effect of Siam weed (*Chromolaena odorata*) and mucuna (*Mucuna utilis*) cover/fallow crops on plant parasitic nematode population with natural bush regrowth as control. They identified eleven genera of nematodes and three, *Meloidogyne*, *Pratylenchus* and *Helicotylenchus species* as predominant across the trial locations. Other important genera present were, *Scutellonema*, *Tylenchorhynchus* and *Rotylenchus species*.

Moreover, *Musa* spp. (that is, banana and plantain) are among the viable fruits presenting the best means of getting foreign exchange by most tropical developing countries like Nigeria. Not only are they revenue yielding, they are important food commodities. They are versatile crops in the tropics that are vended and consumed all over the world as food (Unprocessed) or processed into chips, dodo, flour, etc (Ogazi, 1996; Arias et al, 2003). Due to globalization, they have been exported from the producer countries to large consuming world. The dramatic growth of both the urban and rural populations in Nigeria provides a good opportunity for increased production of the *Musa* spp. for internal consumption and export.

The imperative of export and industrial use of *Musa* spp. demand that good quality fruits are produced. A major constraint to achieving the above goal is plant parasitic nematodes. They attack these plants parts causing crop losses as well as open up the tissues of these plants to bacterial, fungal and viral disease (Pinochet and Stover, 1980; Rotimi, 2003). All these combine to affect growth, yield and the quality of *Musa* spp. in this part of the world. Consequently, *Musa* spp.. is attacked by a complex of numerous species of parasitic nematodes (Bridge, 1993). These nematodes not only display unique geographic ranges but vary in their timing as well as site of attack within the host root system (Sarah et al., 1993). A large number of fungi have been found associated with lesions in roots of *Musa* spp., *Fusarium Oxsponim* being one of the more common species (Mateille and Folkertsma, 1991; De Luca et al., 2012). However, several studies of plant parasitic nematodes have been carried out in different places. Studies in South America and South East Asia indicates that the most important constraints to these *Musa* spp. are soil nematodes, the root-knot nematodes and *Fusarium* moulds coupled with insect infestation (Devos et al., 1978; Coyne et al, 2007; Mobambo et al, 2010; Sayed Abdul Rahman et al., 2014). In Africa, Cameroun, West and Central Africa benefitted immensely from the trade in the *Musa* spp. (banana and plantain) and researchers reports that nematodes are some of the pests of the crop to be studied and controlled (Kamira et al., 2013). At least three nematodes have been mentioned in Cameroun viz: *Radopholus similis*, *Pratylenchus goodeyi* and *Meloidogyne* spp. (Fogain, 2000, 2001).

In parts of Nigeria, very deep insight activities of these nematodes have been obtained. These parasites have been identified as either acto or endo parasitic, and they are either migratory or sedentary. They also showed that research has gone beyond producing a check list of the offending parasites but have also been focused on nematode surveys in various farming systems, geographical locations, habitats and crop hosts including *Musa* spp. (Ogbuji and Ezekwesili, 1977; Obiefuna and Ndubuizu, 1979; Ezekwesili and Anya 1980; Ogbuji, 1983; Rotimi, 2003; Rotimi et al., 2004a, b, 2005, 2006; Olaniyi, 2008; Tanimola et al., 2013).

Speijer et al. (2001) studied plant parasitic nematodes associated with plantain (*Musa* spp..) in Southern Nigeria and their relative importance compared to other biotic constraints. Their results showed that the predominant nematodes found were: *H. multicinctus* which occurred at all 68 sites sampled. *H. pararobustus* occurred at 64% of the sites, *P. coffeae* occurred at 50% of the sites, *R. similis* occurred at 46% of the sites, while *Meloidogype* spp., (second stage juveniles) were found at 68% of the sites. *P. coffeae* was more common in the West and Mid-west of Southern Nigeria, while *R. similis* was more common in the East. They concluded that *P. coffeae* followed by *R. similis* are the major biotic constraints to plantain production and those higher losses are anticipated from these nematodes than by either *Mycosphaerella* spp.., the cause of black sigatoka or *C. sordidus*. Similarly, Elsen and De Waele (2002) noted that out of the nematodes that parasitize bananas throughout the world, that *R. similis* and *P. coffeae* are the most damaging, causing severe yield losses in crops grown commercially and for local consumption.

Inspite of the above studies, the distribution, incidence and characteristics of these parasites in south eastern

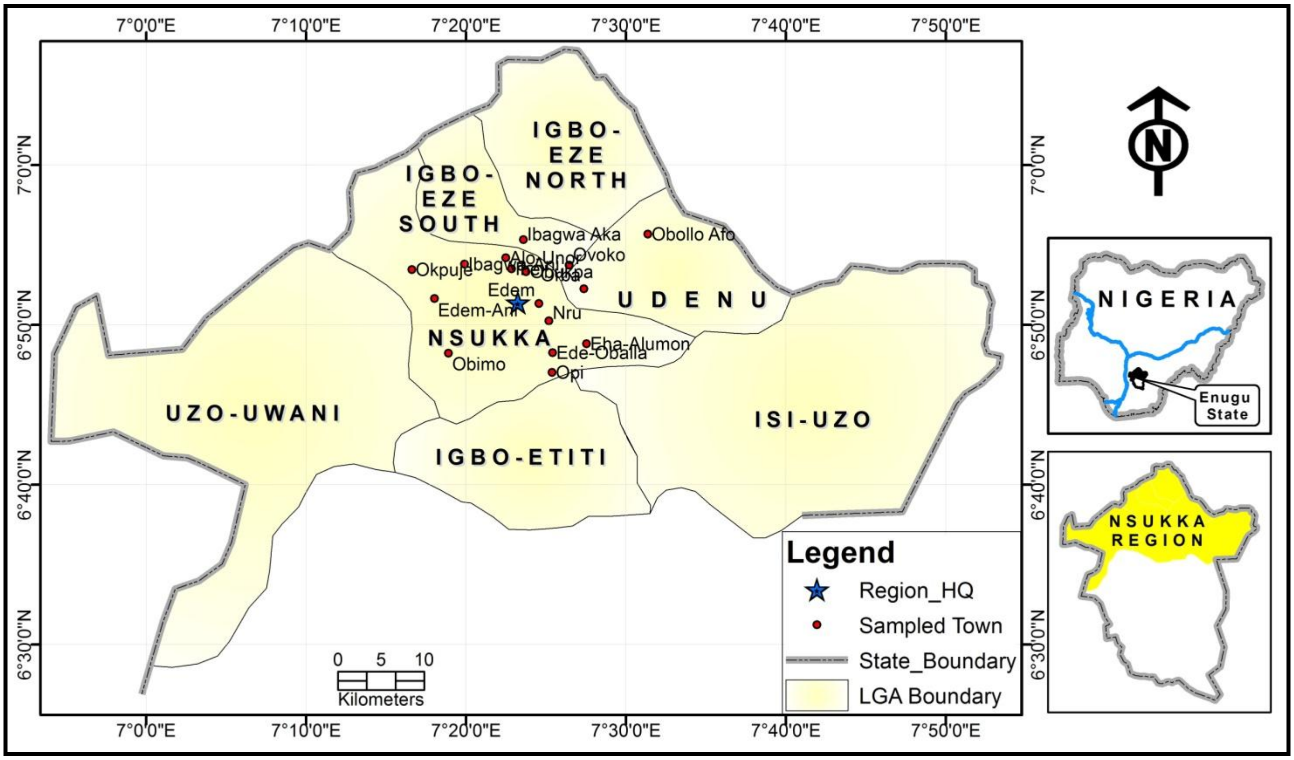

Figure 1. Map of Nsukka Agro-ecological zone showing the sampled areas. Source: Geography Department, University of Nigeria, Nsukka.

Nigeria, especially in Nsukka agricultural ecological zone have not been well documented and that creates a gap in literature and forms the crux of this study. There is need therefore, for adequate knowledge about the distribution of these plant parasitic nematodes of *Musa* spp. and the damages they could cause, including ways of eliminating or eradicating these nematodes for optimum yield of *Musa* spp. in this part of the world.

The aim of this research therefore is to study the distribution of plant parasitic nematodes of *Musa* spp. in Nsukka Agricultural Ecological zone. This can be achieved through the following objectives:

1) To generate information on geo-physical, agricultural and historical information concerning the study area.
2) To determine the physico-chemical properties of the soil as well as their relationships with nematode population extracted from the soil.
3) To profer solutions on ways to control nematode infestation on *Musa* spp. in the study area.

MATERIALS AND METHODS

The study area is Nsukka agricultural ecological zone of Enugu State, Nigeria. It lies between longitude $7°20^{1}$E and $7°29^{1}$E and latitude $6°54^{1}$N and $7°00^{1}$N (Figure 1). The area is predominantly made up of sedimentary formations which fall into two main groups; the Ajali sandstone and Nsukka formation (Reyment, 1965). The general relief of the study area is rugged, which is part of the Udi-Nsukka plateau of the Nsukka-Okigwe cuesta, where remnants of the "African" planation surface are represented by summits of the residual hills (Ofomata, 1985). The main occupation of the inhabitants of the study area is agriculture which is characterized mainly by subsistence agriculture.

Sampling procedure

The research comprised of field surveys and laboratory analyses to study the nematode parasites of *Musa Spp* in Nsukka Agro Ecological zone. For each of the species of the *Musa* spp. that is, banana and plantain, samples were collected from sixteen randomly selected towns in Nsukka Agro ecological zone. Three villages in each town and three sites in each village were visited. These gave rise to total of one hundred and forty four (144) sampling sites each for banana and plantain genotypes. The sampling sites visited were as shown in Table 1.

These villages are the places in Nsukka Agro Ecological zone where *Musa* spp. are produced in large quantities and this informed the choice of these sites.

Pre-tested and validated questionnaire were administered alongside sample collections to randomly selected farmers to generate geo-physical, agricultural and historical information concerning the sample sites, crops and habitats. Five (5) farmers were randomly samples from the 48 sites making a total of 240 farmers sampled for the study.

Root sample collection

Cluster and stratified random sampling methods were used to

Table 1. The sampling sites.

S/N	Towns	Villages 1	Village 2	Village 3
1	Nsukka	Umuoko	Umunengwa	Amokwe
2	Ibagwa-Ani	Ugbene	Eham	Umueze
3	Alor-uno	Udeaguoba	Umuoke	Umuezedim/Ugbene
4	Okpuje	Umueze	Amaho	Umuelu
5	Edem	Obeke	Amaowu	Eziozi
6	Nru	Amadim	Amukpocha	Owerre-enu
7	Eha-Alumona	Amuomumi	Amundi	Odobido Umabor
8	Ede-Oballa	Amama	Umunagu	Amaho
9	Obukpa	Umuezekwe	Ajuona	Agu-Udele
10	Opi	Agbaozalla	Ibeku	Idi
11	Obimo	Ajuona	Umuayiko	Ogwuoda
12	Obollo	Iheakpu	Umutenyi	Ogwu
13	Orba	Owerre-Ezeoba	Amube-Oham	Amuji lagwu-Oham
14	Ovoko	Ajuona	Amachara	Amaebo
15	Iheaka	Likke	Iheaka	Obollo
16	Ibagwa-Aka	Ndoke	Ikolo	Amebo

collect root samples from each site. Fresh root samples were collected from seven randomly selected communities out of the sixteen communities earlier surveyed. The root samples were washed with tap water to remove sand and debris. 20 cm length or root was cut into smaller pieces and gently teased with pestle in laboratory porcelain mortar. The mashed root was washed into a muslin cloth and placed on the plastic dish following the modified Baermann's method. The experimental set up was allowed to stand undisturbed for one day (overnight). The debris was removed and the extracted water allowed to sediment. Clear excess water was decanted. An aliquot (5 µg) of the nematode suspension was pipetted into an improvised counting dish with an aid of a micro pipette. The suspension was kept shallow. The suspension on the counting dish was placed on the dissecting stereo – microscope using the lowest convenient magnification and with under stage lighting to enable the nematodes to be seen more clearly. The nematodes (adults or juveniles) were counted and recorded. The nematodes were also captured with mounted digital photographic camera which was later processed in a colour laboratory. The pictures were scanned and further processed using the computer software called Adobe photo shops. The nematodes were identified and classified with help of a practical plant nematology field and laboratory guide as well as assistance from experts.

Soil sample collection

Soil samples were collected at a depth of 20 cm from each of the locations where plant root samples were collected. The three soil samples collected from each site were bulked together into a composite sample for each site. These gave rise to a total of forty eight composite samples, each for banana and plantain genotypes. The sample collection was done the year 2012.

Laboratory soil analyses

Laboratory soil analyses were carried out to determine the physical and chemical characteristics of the soil samples. Extraction of nematode larva from the soil was carried out using the modified Bearman's funnel Laboratory Root analyses were also carried out for the fixing and extraction of nematode eggs, and for identification and classification of nematodes.

Data analysis

All data collected were subjected to analysis of variance (ANOVA) using General statistics (GENSTAT) Discovery Edition. Means were separated using the least significant Difference (FLSD) at 5% probability level. Regression analysis and other descriptive statistics were performed with the aid of the statistical package for social sciences (SPSS) version 16.

RESULTS

The result of the study shows that the farmers in the study area cultivate mostly on homestead habitat followed by farmland which is just a few distance from the backyard and very little production goes on in the wild. There is concentration of *Musa* spp. around the homestead, within the surveillance of the farmer, probably to avoid volunteer harvesting by thieves as informed by the respondent farmers. From meteorological information of the study area in the year 2012, rainfall was very high in August and September, high in June, moderate in April, May and July and basically no rains in January, February and December. During the peak of the sample collection in June, the relative humidity was very high at approximately 81%. Average soil temperatures for the month was within the normal range. Average volumetric soil water content (m/sec), was moderate and so on.

The mechanical and chemical analysis of soils of the study area under the particle size analysis showed that

Table 2. Rotated component matrix of principal component analysis for the physical and chemical properties of the soil samples.

Physical and chemical properties	Components			
	1	2	3	4
Clay	-0.357	0.029	0.712	0.051
Silt	-0.059	-0.477	0.360	0.051
Fine sand	0.004	0.050	0.903	0.034
Coarse sand	0.140	-0.020	**-0.965**	-0.078
H_2O	0.861	-0.066	-0.312	-0.118
KCL	0.726	0.204	-0.493	-0.075
Carbon	0.020	0.824	-0.012	0.152
Organic matter	0.019	**0.949**	0.104	0.094
N	0.382	-0.240	0.130	-0.621
Na^+	0.333	0.466	0.511	-0.273
K^+	0.564	0.730	-0.-49	-0.084
Ca^{2+}	0.822	0.452	0.254	0.083
Mg^{2+}	0.565	0.757	0.125	-0.093
Cation exchange capacity	0.615	0.410	0.447	0.232
Base salt%	0.556	0.503	-0.490	-0.115
H^+	0.087	0.080	0.059	**0.773**
Phosphorus	**0.871**	0.105	-0.215	-0.106

Total variance explained by the four components is appraximately 79.60%

the soils for the communities fall under (3) textural classes viz: Sandy soil, sandy-loam soil and loamy soil. The percentage soil organic matter and organic carbon were generally low. Most of soils of the communities were acidic in reaction whether the pH value was measured in H_2O or in NaCl (5.8-8.7 for pH value in H2O and 5.2-7.5 for pH value in NaCL). The contents of the major elements in the soil (N, exchangeable baases- Na^+, K^+, Ca^{2+}, Mg^{2+} and exchangeable acidity-H^+) were rather low. The percentage base salt was quite high with most soils saturated ranging above 80%. Percentage base salt saturation was quiet high with most soils saturation ranging above 80%. Total phosphorus was moderately high with most soils measuring above 30ppm. The Cation Exchange Capacity (CEC) of the soils was rather low with 14.0 m/100 g as the highest record.

Relationship between nematode density and some geo-physical and agricultural information of the sampling sites

The extraction of highest nematode larval density in the homestead compared with farmland and wild habitats, flat topography compared with slightly sloppy or steep slope topography and dark brown soil compared with brown and light brown soils could be due to the fact that they receive more organic manures which apart from increasing plant vigour also provide a conducive environment for nematodes. Dark brown colour in soils mostly confers a measure of fertility and also confirms that there was a judicious amount of organic matter (humus) in the soil which in turn favours nematodes habitation. Rotimi (2003) also obtained a similar result.

The rotated component matrix was used to determine what the set of components represents. The first component correlated highly with phosphorus. The second component correlated highly with organic matter. The third component correlated highly with coarse sand and the fourth component correlated highly with H^+ and this suggests that we can focus on these four components in further analysis. The percentage of variance accounted for by the four components is 79.6% approximately 80%. This implies that the four components explain nearly 80% of the variability in nematode density attributable to the original seventeen variables (Table 2).

Although the variables selected from the results of the PCA suggests using phosphorus, organic matter, coarse sand and H^+ but for further analysis, silt was included as well because it was the only variable that was selected in a stepwise multiple linear Regression Analysis that includes all the seventeen variables. The regression method selected only silt as the significant factor to nematode density. The model built adequately based on the fact that the p-value of the F- test conducted on the adequacy of the model is less than 0.05. The model has r-square of 0.392, which implies that about 39% of the variation in nematode density was attributed to silt (Table 3).

Table 3. Stepwise multiple linear regression result for Nematode density.

Regression equation					
Density			21504.00 - 1126.50 × silt		
R-square			39.2%		
		Analysis of overalll equation			
Source	**DF**	**SS**	**MS**	**F**	**P-Value**
Regression	1	1.269E8	1.269E8	9.009	0.010
Residual error	14	1.972E8	1.409E7		
Total	15	3.241E8			
		Analysis of individual predictors			
Predictor	**Coefficient**	**SE(ccoefficient)**	**T**	**P-value**	
Constant	21504.00	3325.283	6.467	0.000	
Silt	-1126.50	375.313	-3.001	0.000	

Df, Degree of freedom; SS, sum of squares; MS, means square; F, F-test value.

From the data collected on the distribution of plant root nematodes in the study area (indices of diversity and dominance); in *Musa* spp., the diversity indices of these parasites were derived using Shanon- Weiner model with the formular (n/N) (LN(ni/N). The outcome shows that there was high diversity index (1.7497) for the whole plant parasitic nematodes in the study area and *Meloidogyne* spp. is the nematode with the highest diversity index.

Again from the distribution of nematode species in roots samples of *Musa* spp. from sixteen towns in Nsukka Agro Ecological zone, the survey for the parasites show that altogether, eight nematode spaces from six genera were isolated. The result of the chi-square test for equality of proportions (density) of the species clearly indicates that the proportions of nematodes species are significantly different ($p < 0.05$). From the result, *Meloidogyne* spp. was taken as the dominant nematode specie in Nsukka Agro Ecological zone. A study of Table 4 shows that *Meloidogyne* spp. was the most frequently encountered nematode (34.4%) while *Scute vonema* spp. was the least abundant specie in the study area. This distribution is also shown in Figure 2.

Table 5 brings together the soil nematode larval density and the root nematode egg mass density. It was shown that in all cases of recorded densities, nematode egg mass was always higher than the nematode population density (adult and larva). Again since the values of the slope in the resulting regression equation are approximately zero, there is no significant linear relationship (no effect) between nematode population density and mean root weight of *Musa* spp*.*

DISCUSSION

Like most *Musa* spp. plants in the tropics, this study has shown a high level of parasitization by root nematodes and indicates that of all the prevailing parasites, *Meloidogyne* was the most prevalent nematode species extracted from the studied sited. This is folowed by *R. similis, Rotylenchus* spp*., H. milticinctus, Pratylenchus* spp. etc. This is supported by earlier reports by Lowe (1992) and Speijer et al (2001a) which states that *H. multicinctus* and *Meloidogyne Musa* spp. are ubiquitous in Nigeria. Rotimi et al. (2005) recorded that *P. coffea, R. similis, H. multicinctus* and *Meloidogyne* spp. were the key nematodes in Southern Nigeria. *Meloidogyne* is the specie considered to be of significant effect in Nsukka agro-ecological zone since it was recorded as the highest dorminant and diverse specie.

Musa spp. production in Nigeria is predominantly practiced by peasant farmers who cannot normally afford high cost dependent inputs like fertilizers, herbicides, synthentic pesticides including nematicides, improved planting materials, etc. The farmers in the study area cultivate mostly on homestead habitat which is just a few distance from the backyard and very little production goes on in the wild. Concentration of *Musa* spp. around the homestead, within the surveillance of the farmer is probably to avoid volunteer harvesting by thieves as informed by the respondent farmers. Similar finding was reported by Swennen and Vuylsteke (1998) who found that crop production is normally on a small scale field production or in backyard gardens. This result also indicates that *Musa* spp. are not planted in an intensive and organized pattern like other food crops such as yam, cassava, etc, which have more regular pattern of production.

The smallholder' lands are often marginal and due to rising population pressure which leads to shortened fallow period, there are the consequences of declining soil fertility and high soil acidity. This is evident from the high pH values obtained from the chemical soil analysis

Table 4. Distribution of nematode species in roots samples of *Musa Spp* from seven randomly selected towns in Nsukka Agro Ecological zone.

Nematode specie/0.05 mL aliquot	Population density	Percentage population (%)
Meloidogyne Spp.	288	34.4
Radopholus similis	201	24.0
Rotylenchus reniformis	86	10.3
Rotylenchus borealis	102	12.2
Pratylenchus goodeyi	26	3.0
Pratylenchus coffeae	49	5.9
Helicotylenchus multicintus	72	8.6
Mesocriconema specie	13	1.6
Total	**837**	**100.0**

Chi-Square test value is 592.63 with p-value of 0.0000.

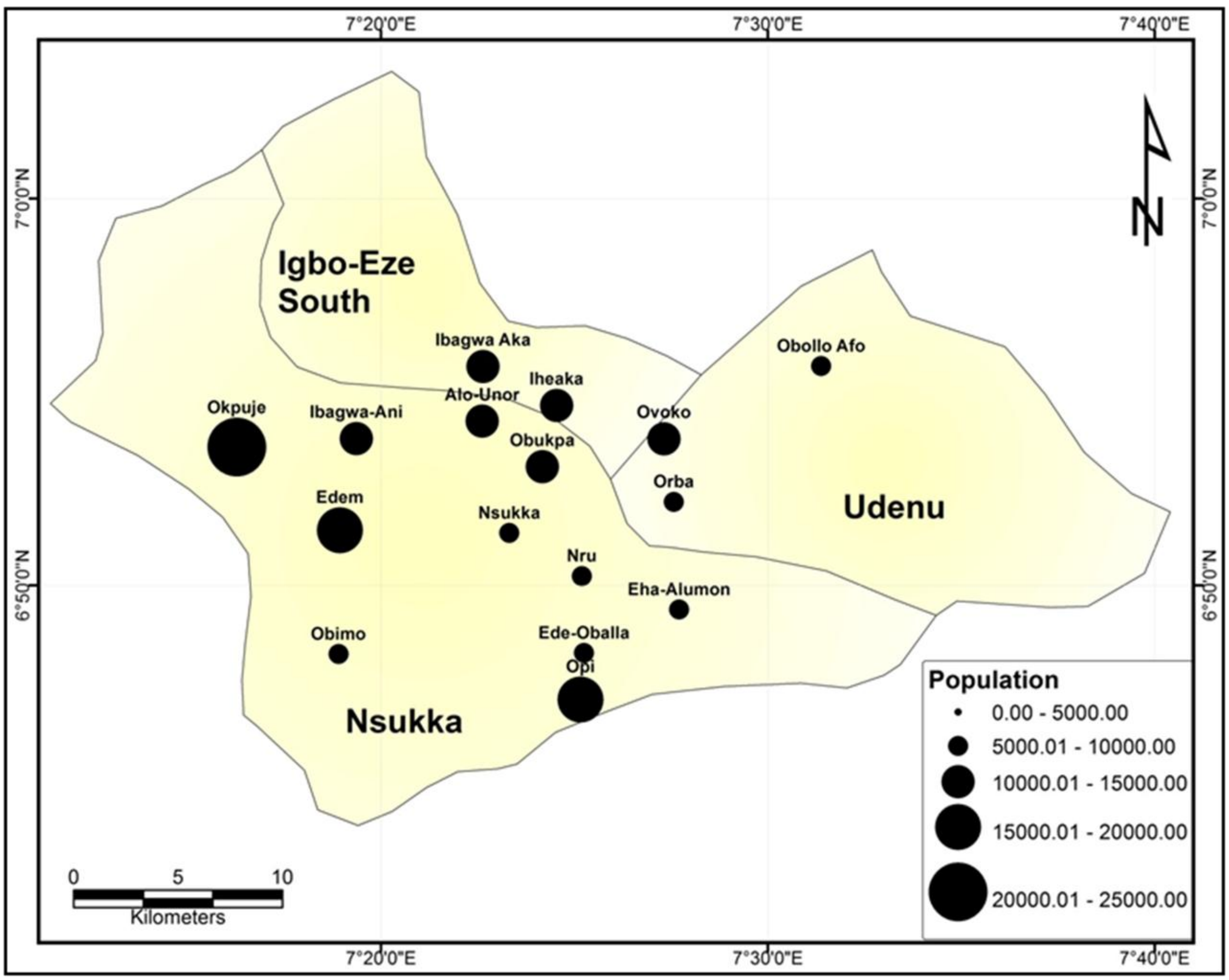

Figure 2. Distribution of nematode species in roots samples of *Musa spp* from seven randomly selected towns in Nsukka Agro Ecological zone. Source: Geography Department, University of Nigeria, Nsukka.

showing the soil samples as acidic in nature. Nematodes do not thrive well in acidic soils. This was evident in the negative correlation between nematode population and soil pH obtained in the study. Moreso, poor fertility status

Table 5. The population density of nematode larva and egg extracted from rhizosphere soils and the roots of *Musa spp*. in Nsukka agro-ecological zone.

Towns	Nematode larvae density	Nematode egg mass
Alor Uno	10,500	30,840
Ede Oballa	8,490	25,680
Edem	15,660	42,300
Eha Alumona	9,510	31,950
Ibagwa-Aka	14,010	30,330
Ibagwa-Ani	17,010	39,330
Iheaka	10,500	29,250
Nru	6,840	34,500
Nsukka	8, 820	26,250
Obimo	8,340	23,550
Obollo Afor	8,340	35,070
Obukpa	10,500	31,950
Okpuje	24,510	35,820
Opi	17,340	31,890
Orba	9,330	20,670
Ovoko	11,160	37,950

of soil aggravates nematode activities on *Musa* spp. (Rotimi et al., 2004a, b).

High nematode population pressure in the soil obtained was in line with Rotimi et al. (2004) and Olaniyi (2011) who noted that with increasing moisture level in the soil, the population density of *R. similis* increased and was highest at the field capacity. This indicates that at the peak of the rainy season, nematodes population would be high and this was the situation at the peak of the sample collection in this research. This result was also confirmed by Sehgal and Guar (1995) who reported that soil moisture level is an important factor that modulates *R. reniformis* activities and densities, although there is a need for verified and controlled environmental studies under different field conditions before management decisions are taken. High population densities of nematodes were extracted from *Musa* spp. roots as well as the soils from the rhizosphere of the roots. Rotimi et al., (2006) recovered high population densities of *R. similis* from roots of suckers.

Regression analysis on the effect of nematode number on weight of plant root showed a negative correlation. This implies that nematode egg load is not strongly associated with root weight decrease. Rotimi et al. (2005) reported that nematodes caused 45% root weight reduction. However, Olaniyi (2014) recorded a strong correlation between nematode densities in the rhizosphere soil and root damage and thus concluded that loses due to nematodes have often been underestimated since the soil densities were not often taken into consideration. This research work investigated both nematodes densities in plant roots as well as soils in the rhizosphere of the roots which is in aggrement with Hooper (1990).

CONCLUSION AND RECOMMENDATION

The results from the survey of the distribution of plant-parasitic nematodes of *Musa* spp. in Nsukka agricultural ecological zone, Enugu state, Nigeria reveal that the plant parasitic nematodes are widely distributed on *Musa* spp. in the sixteen (16) communities in the study area, probably through the use of infected planting materials. Also revealed are 8 genera of nematode species; *Meloidogyne* spp., *R. similis, R. reniformis, R. borealis, P. goodeyi, P. coffeae, H. multicinctus* and *Mesocriconema* spp. The most important in relation to root damage were *Meloidogyne* spp., *R.similis* and *R.borealis. Meloidogyne species* had the highest population density. There exist slight changes in the types, distribution and populations of nematodes from one part of Nsukka to another which might be due to environmental factors, soil physio-chemical properties and agronomic practices (Olaniyi, 2014). The effect of nematode damage on production will increase progressively with each follower crop cycles and become more pathogenic as nematodes population continues to build up if no precautionary measures are taken.

Potassium is strongly implicated in heavier bunch yield (Salau et al., 1992) and improved crop quality (Treshow, 1970). Therefore, attempts to develop potassium efficient genotypes of *Musa* spp. could result in improved materials that would combine nematode resistance with high quality yield. Potassium might be an important key element in nematode control in *Musa* spp. as it improves the rigidity of plant organs, increases resistance of plants to diseases and helps plants to withstand environmental stresses such as adverse weather and temperature as well as low soil fertility (Treshow, 1970; Bruehl, 1987; Kirkpatrick et al., 1994; Coyne and Tenkouano, 2005). The farmers should form the habit of applying manures to their crops since it is now an established fact that the plants could benefit from inputs of fertilizers or manure. Optimum level of organic mulching could suppress nematode population and is an attractive approach to the management of plant parasitic nematodes ecology in an integrated management concept (Sarah, 1989).

This study advocates the use of clean and healthy planting materials, possibly tissue culture plants. Quarantine and pre-planting treatments should be observed to prevent emerging nematode parasites surge. IITA with mandate on the crop should hasten efforts in the development and distribution of nematode resistant or nematode tolerant cultivars by classical breeding. The principles of crop rotation in nematode management should be upheld (Sarah, 1989; Gowen et al, 2005). The use of intercropping with certain nematode antagonistic crops such as *Tagetes* spp., Sesame, mustard, etc, has been reported to reduce root knot and *R. reniformis* nematodes. African marigolds (*T. erecta*) have been shown to inhibit the reproduction of root knot and reniform nematodes. The use of trap or antagonistic crops such as crops like *Crotalaria spectabilis* which allows invasion by *Meloidogyne* spp. but does not support development can serve as good trap crops (Hill and Waller, 1999).

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Water use efficiency of controlled alternate irrigation on wheat/faba bean intercropping

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Water supply is limited worldwide and there is a need for saving of water in irrigation. In this study, two irrigation methods, that is, conventional irrigation (CI), alternate irrigation (AI, alternate watering on both sides of the row), and three planting patterns, that is, sole wheat, sole faba bean and wheat/faba bean intercropping were applied on root box experiment. Results showed that the yields of intercropping with AI generally increased compared with sole cropping. Sole faba bean and wheat/faba bean intercropping with AI significantly increased the root-shoot ratio compared to all the other treatments. P_n in AI was not reduced significantly when compared to that of CI at the same planting pattern. However, T_r and G_s in AI was significantly lower than that of CI for sole wheat, sole faba bean and wheat/faba bean intercropping. Maximum biomass accumulation was obtained in the CI treatment, but severe water deficit led to a less reduction in the AI treatment, such reduction was much smaller under AI and therefore the higher water use efficiency was obtained. In conclusion, AI is an effective and water-saving irrigation method in wheat and faba bean production especially in intercropping system, and may have the potential to be used in the field.

Key words: Yield, irrigation, water use efficiency, wheat (*Triticum aestivum*), Faba bean (*Vicia faba*).

INTRODUCTION

Intercropping, the agricultural practice of cultivating two or more crops in the same area at the same time, is an intensive management for crop production which aims to match efficiently crop demands to the available growth resources and labor. It is relatively common in tropical and temperate areas, because of the effective utilization of water (Xu et al., 2008), nutrients (Xia et al., 2013; Zhang and Li, 2003) and solar energy (Yang et al., 2014). The majority of intercropping systems involve legume/cereal combinations, interspecific facilitation occurs when one plant species enhances the growth of another plant species and has been observed mainly in legume/cereal systems (Li et al., 1999, 2001), and most of the research that has been conducted on intercropping has therefore focused on the legume-cereal intercropping, a productive and sustainable system (Ghosh et al., 2009; Lithourgidis

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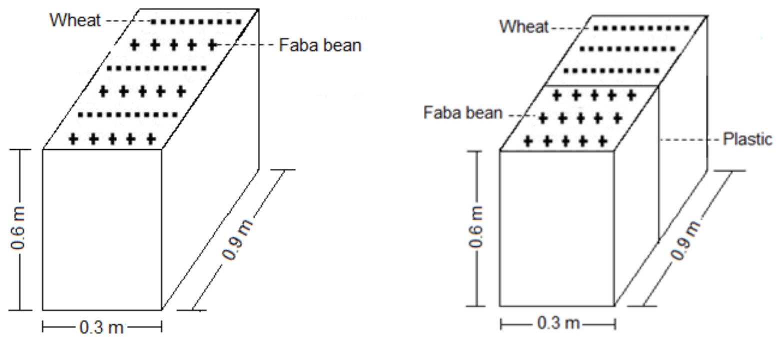

Figure 1. A schematic diagram of root box for wheat and faba bean controlled alternate irrigation.

et al., 2011; Tosti et al., 2010). In China, half of the total grain yields was produced with intercropping (Zhang and Li, 2003), wheat/faba bean intercropping system in particular was one of major planting pattern in northwestern regions. In general, intercropping has been shown to be more productive than sole cropping. However, combinations of certain crops result in increased competition among the components. This results in reduced yields, which may make some crop species unsuitable for intercropping. Increased competition especially for water may be ultimately leading to changes in crop productivity levels.

Drought is one of the most important environmental factors limiting growth and yield of crops (Chaves et al., 2003). In the traditional agricultural irrigation, yield increase was mainly attained from the amounts of water used in irrigation satisfying the biological characteristics of water demand (Deng et al., 2002). Enhancing water use efficiency, both under rain-fed and irrigated agriculture is a high priority for agricultural improvement in developing countries (Canone et al., 2015; Melkonyan, 2015; Ronaldo et al., 2015). Thus, new irrigation strategies must be established to use the limited water resource more efficiently. In recent years, the concept of alternate partial root-zone irrigation (APRI) or partial root-zone drying (PRD) has been raised and attracted considerable interest (Kang et al., 1997; Davies et al., 2000; Kang and Zhang, 2004).

This technique was derived from earlier split-root studies (Blackman and Davies, 1985) and requires that approximately half of the root system be always exposed to drying soil while the remaining half is irrigated as in full irrigation. The wetted and dried sides of the root system were alternated in a frequency according to crops growing stages and soil water balance. This could result in the concentration increasing of abscisic acid (ABA) in the xylem flow from roots to leaves, triggering the closure of stomata (Tardieu et al., 1993; Zhang and Davies, 1990), and decreasing the transpiration of crops. Water management under PRD irrigation focuses on efficient use of limited soil water and increasing crop water-use efficiency. Root growth is critical for crops to use soil water and obtain high yield under water deficit conditions (Robertson et al., 1993). The effect of this irrigation mode on increasing WUE and maintaining yield has been extensively verified (Davies and Hartung, 2004), it is hypothesized that these same benefits may be obtained in wheat/faba bean intercropping. However, the effects of enhanced WUE on wheat/faba bean intercropping have not been investigated.

In wheat/faba bean intercropping system, the conventional irrigation scheme was often designed only according to the water demand of a main crop in deficient growth stage, it often caused the mismatching of water supply and demand for the other crop. For the sake of solving the problem, the authors explored a new irrigation method that based on limited water supply but could meet the water demand on both wheat and faba bean, might be an effective way to decrease the irrigation quota and increase the WUE of wheat/faba bean intercropping system. The experiment was designed to test the effect of controlled alternated irrigation on the yield, root-shoot ratio, physiological response and WUE of wheat/faba bean intercropping. The objective of the study was to compare AI with different planting pattern on wheat and faba bean plants in terms of plant root and shoot growth, shoot physiology and WUE.

MATERIALS AND METHODS

Plant material and experimental conditions

The experiment was carried out under glasshouse conditions at the plant growth unit, Gansu Agricultural University, Lanzhou (Latitude 36°03'N, longitude 103°49'E), China. It was conducted from January to July 2008. The wheat were sown on 25 March and harvested on 15 July, while the faba bean were sown on 28 March and harvested on 22 July. Wheat and faba bean plants were grown in root box (900 mm in length, 300 mm in width and 600 mm in height), three strips comprising 3 rows of wheat and 3 rows of faba bean constituted a root box, the inside of the root box was evenly separated into two containers (sole cropping) with plastic sheets such that water and nutrients exchange among the containers was prevented (Figure 1).

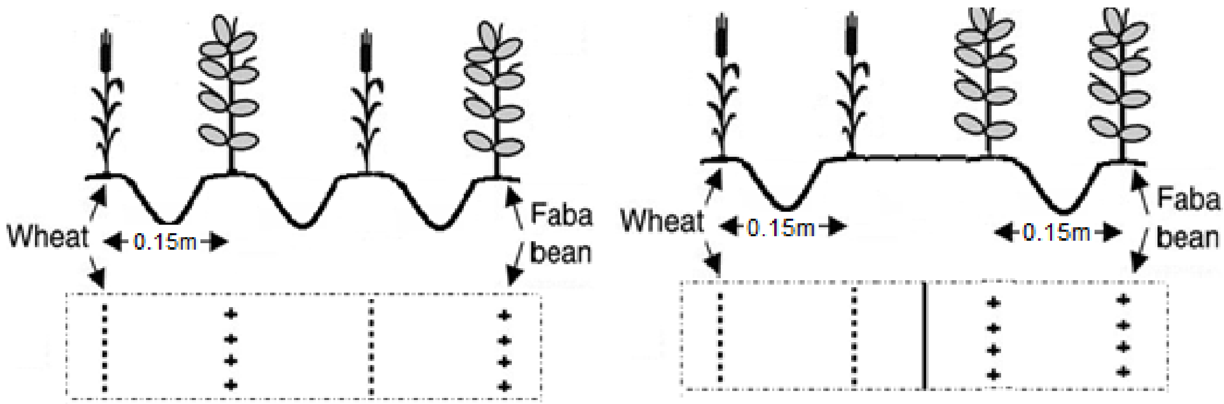

Figure 2. Layout of wheat/faba bean intercropping and sole cropping for controlled alternate irrigation in root box experiment.

The row spacing of wheat and faba bean were all 150 mm. Each root box consisted of 3 rows with 45 plants for wheat and 3 rows with 9 plants for faba bean (Figure 2). Root boxes were filled with heavy loam soil with a bulk density of 1.17 g dried weight cm^{-3} and field capacity of 24.3% (g water g^{-1} wet weight). Soil was sieved by 2 mm mesh and mixed with NPK-complete fertilizer to keep soil structure, nutrition and soil microorganism homogeneous at the beginning. In addition, a PVC tube (2 cm in diameter) with holes was installed in each root box to supply irrigation water to prevent surface soil hardening from the irrigation and reduce evaporation.

Treatments and experimental design

Experimental design was a split-plot with three replicates in which the main plot treatments were alternate irrigation (AI) and conventional irrigation (CI). CI which every row was irrigated during each watering, and AI which one of the two neighboring furrows was alternately irrigated during consecutive watering. Each root box was considered as a block to randomly allocate the above six treatments in a randomized complete block design (RCBD) with nine replications. Sub-plot treatments consisted of sole wheat seeds (*Triticum aestivum* L., Yongliang No. 4), sole faba bean (*Vicia faba* L. *cv.*, Lincan No. 5) and wheat/faba bean intercropping.

At the age of 3 weeks, plants were chosen for uniformity and relatively even root distribution among the root boxes and subjected to controlled soil water content. Before the soil water was controlled, soil water regimes in all root boxes were kept the well-watered (60% of their field capacity). Irrigation was strictly controlled when soil water content reduced to or near to the lower limit of soil water content (55% of their field capacity). The amount of water used for each plant was recorded. The root box size was such that interval of 5 days was enough to keep the soil water content above the designed levels. Crop water consumption (evapotranspiration) and the amount of irrigation were calculated from the root box water balance.

Measurements

At the end of the experiment, wheat and faba bean were harvested for their root dry mass, shoot dry mass, and the final grain yields for each individual plant were also recorded. Land equivalent ratio (LER) was used to evaluate the success of intercropping, the total LER is defined as the total land area required under monoculture to give the yields obtained in the intercropping mixture. It is expressed as:

$$LER = \frac{Y_{WI}}{Y_{WM}} + \frac{Y_{FI}}{Y_{FM}} \quad (1)$$

Where Y_{WM} and Y_{FI} are grain yields of wheat in monoculture and intercropping (kg·ha-1), respectively; Y_{MM} and Y_{FI} are grain yields of maize in monoculture and intercropping, respectively (kg·ha^{-1}). WUE was calculated for each plant as the harvest yield divided by the total amount of water actually irrigated.

During the soil drying period, the photosynthetic rates, transpiration rates and stomatal conductance of all plants were measured at critical growth stage using a photosynthesis analysis system (CI-301PS, CID, USA). These measurements were made on fully expanded leaves facing the sun at about 9:00 to 11:00 h.

Data analysis

Root, shoot growth and physiological data were analyzed with standard split-plot analysis of variance (ANOVA) techniques, with irrigation method as the main factor and planting pattern as the sub factor. The least significant difference (LSD) test was used to compare significant differences between treatment means. The level of significance was set at 5%.

RESULTS

Yield response

Intercropping of wheat and faba bean showed some advantage in terms of grain yields, and LER based on grain yield at maturity were greater than 1.0 whether AI or CI (Table 1). The effect of irrigation method on total yield of sole faba bean was not significant (p>0.05), and the response of intercropped faba bean with AI was also not significant. However, the yields of intercropping with alternate irrigation generally increased compared with sole cropping. The yield of intercropped wheat was 62.75% of sole wheat, and intercropped faba bean was 52.77% of sole faba bean in AI.

Analysis of variance showed that planting pattern has significant effect on grain harvest index. The harvest

Table 1. Yield of wheat/faba bean intercropping subjected to controlled soil drying.

Treatment		**Yield(g)**		**Harvest index**		**LER**
		Wheat	**Faba bean**	**Wheat**	**Faba bean**	
A	S	4.59	16.44	0.3600	0.4214	1.16
	I	5.76	17.35	0.3942	0.4459	
C	S	5.17	16.51	0.3799	0.4159	1.11
	I	5.81	17.97	0.3905	0.4368	
Significance test (*P* values)						
Irrigation method		NS	NS	NS	NS	
Planting pattern		**	*	*	*	
I×P		NS	NS	*	*	

Note: A-alternate irrigation, C-conventional irrigation, S-sole cropping, I-intercropping, I×P-Interaction effects between irrigation method and planting pattern; NS refers to no significant difference between treatments at 0.05 levels, *Significant difference between treatments at 0.05 levels, **Significant difference between treatments at 0.01 levels; The same as follows.

Table 2. Dried root mass and root-shoot ratio of wheat and faba bean plants subjected to controlled soil drying.

Treatment		**Wheat**			**Faba bean**		
		Shoot dry weight ($g \cdot plant^{-1}$)	**Root dry weight ($g \cdot plant^{-1}$)**	**R/S ratio**	**Shoot dry weight ($g \cdot plant^{-1}$)**	**Root dry weight ($g \cdot plant^{-1}$)**	**R/S ratio**
A	S	12.75	2.10	0.165	13.99	3.23	0.231
	I	14.61	2.54	0.174	14.15	3.13	0.221
C	S	13.61	1.65	0.121	13.66	2.70	0.198
	I	14.88	2.26	0.152	15.58	2.91	0.187
Significance test (*P* values)							
Irrigation method		NS	NS	*	NS	*	NS
Planting pattern		**	*	NS	*	NS	*
I×P		NS	NS	*	*	*	**

index of intercropped wheat and faba bean was increased by 2.27 and 5.42% than corresponding sole cropping. No significant difference in harvest index was observed between AI and CI.

Root and shoot response

Table 2 shows root establishment in the soil as a function of the different planting pattern and irrigation ways of controlled soil drying. The shoot and root dry weight of intercropped wheat was significantly increased by the root intermingling compared with sole cropping with either CI or AI. However, this did not result in significant differences in the root-shoot ratio under different planting pattern.

Generally, shoot dry weight is not significantly different between the two irrigation methods. AI slightly influences the above ground growth of wheat and faba bean plant, root dry weight in AI was more than that of CI, and the difference between AI and CI was significant for sole faba bean and wheat/faba bean intercropping. Soil drying inhibited root growth, but in this experiment, the root-shoot ratio was increased, suggesting that shoot growth is more sensitively inhibited than roots. It is indicated that AI in sole faba bean and wheat/faba bean intercropping significantly increased the root-shoot ratio compared to other treatments.

Physiological responses

For monitoring change of photosynthesis rate (P_n), transpiration rate (T_r) and stomatal conductance (G_s) of the leaves, sunny and cloudless days were chosen with a portable photosynthesis system (CI-301PS, CID, USA) at about 9:00 to 11:00 h on May 14, May 29 and June 10,

Table 3. Photosynthesis rate, transpiration rate and stomatal conductance of wheat and faba bean subjected to controlled soil drying.

Date (month/day)	Treatments		P_n		T_r		G_s	
			Wheat	Faba bean	Wheat	Faba bean	Wheat	Faba bean
5/14	A	S	6.86	8.54	2.85	1.55	0.85	10.54
		I	7.58	8.24	3.25	1.68	0.91	11.03
	C	S	6.85	9.23	3.23	1.69	1.11	10.56
		I	6.83	8.21	3.26	1.87	0.75	12.56
5/29	A	S	8.51	10.21	5.14	2.11	2.31	11.56
		I	8.98	11.12	5.58	2.33	1.84	13.23
	C	S	8.57	15.54	4.95	2.56	1.85	13.56
		I	9.5	14.25	4.86	2.45	1.92	11.65
6/10	A	S	9.87	16.12	7.56	2.89	2.61	16.25
		I	9.55	15.56	7.23	2.87	2.53	16.56
	C	S	9.52	17.13	7.14	2.98	2.44	16.29
		I	10.24	15.56	6.88	2.87	2.68	17.11
Significance test (*P* values)								
Irrigation method			NS	NS	*	*	*	*
Planting pattern			*	*	NS	*	**	*
I×P			NS	NS	*	*	*	*

P_n-Pnotosynthesis rate (umol/m^{-2}s^{-1}), T_r-Transpiration rate (mol/m^{-2} s^{-1}), G_s-Stomatal Conductance (mol/m^{-2}s^{-1}). The data were measured at 9:00 to 11:00 h for each time in sunny and cloudless days. The dates of the measurements were in the middle of two irrigations. Each value is a mean of three replicates.

2008 (Table 3). Four youngest mature leaves that were fully exposed to sun were chosen for such measurement of each treatment. P_n in AI was not reduced significantly when compared to that of CI at the same planting pattern. However, T_r in AI in three measurements for sole wheat and sole faba bean was also significantly lower than those of CI. It should also be noted that G_s in AI in three measurements showed the similar changes as T_r for sole wheat, sole faba bean and wheat/faba bean intercropping. AI did not lead to a leaf water deficit that might havecontributed to growth and stomatal regulation. When compared to CI, AI did not significantly inhibit leaf photosynthesis, but did significantly restrict stomatal opening, especially for sole faba bean and wheat/faba bean intercropping. As a result, WUE in AI was significantly higher than that of CI in the respective measurements at the same planting pattern.

Water use efficiency (WUE)

WUE calculated as the yield or biomass production per unit amount of water consumption was substantially improved in these treatments. Improved irrigation methods (AI) can significantly

Table 4. Total water consumption, biomass and water use efficiency (WUE) of wheat and faba bean plants subjected to controlled soil drying.

Treatment		Dry biomass (g)	Water consumption (kg)	WUE_y (g/kg)	WUE_b (g/kg)
	Sole wheat	12.23	17.48	0.263	0.700
A	Sole faba bean	14.55	19.53	0.842	0.745
	Intercropping	15.09	16.36	0.706	0.922
	Sole wheat	12.44	26.76	0.193	0.465
C	Sole faba bean	10.94	27.84	0.593	0.393
	Intercropping	15.74	27.26	0.436	0.577
Significance test (*P* values)					
Irrigation method		*	**	*	*
Planting pattern		**	NS	*	NS
I×P		NS	*	NS	NS

improve wheat/faba bean intercropping water use efficiency (Table 4). Compared to CI, AI increased WUE_y and WUE_b by 35.94, 41.94, 61.90% and 50.53%, 89.58%, 59.71% for sole wheat, sole faba bean, wheat/faba bean intercropping, respectively; However, wheat/faba bean intercropping significantly improved WUE_b than corresponding sole wheat and sole faba bean. As the root growth data in Table 1 have suggested, such drying and rewetting methods with a moderate root-shoot ratio were possibly too aggressive in terms of water saving.

DISCUSSION

Previous studies have shown the yield advantage in legume/non-legume intercropping systems. The results were reported for mixed cultures of wheat and faba bean (Agegnehu et al., 2008; Tosti and Guiducci, 2010; Barker and Dennett, 2013), wheat and field bean (Haymes and Lee, 1999), barley and faba bean (Agegnehu et al., 2006), maize and faba bean (Li et al., 1999; Mei et al., 2012) and wheat and lentil (Akter et al., 2004). In this experiment, there was also an advantage in wheat/faba bean intercropping over monoculture as the LER was above 1.0 under different irrigation method. The total yield of wheat/faba bean intercropping was also significantly higher than sole wheat and sole faba bean respectively.

A number of experiments have reported that alternate irrigation (AI) maintain a reasonable crop yield and save irrigation water (Kang et al., 2002b; Liang et al., 2013; Tang et al., 2005; Ye et al., 2013; Zegbe et al., 2004). It has been proved to be an effective irrigation way for many crops and in many areas (Kang and Zhang, 2004). In our study, AI method on wheat/faba bean intercropping could improve WUE without a significant decrease of photosynthetic rate and biomass, which is in agreement with some earlier research on AI (Du et al., 2013).

Generally, aboveground and belowground architecture of plants usually influences plant's relative competitive ability (Akanvou et al., 2001; Dingkuhn et al., 1999). The response of different cropping system to irrigation method, however, was not identical with intercropping being more sensitive than sole cropping. The soil drying signal from the partially irrigated root system could accouter for the reduced vegetative growth observed in the AI treatments plants. This study has shown that green gram for AI invested proportionally more of its photosynthetic resources into yield and biomass production per unit of water transpired, whereas faba bean invested more heavily in root production. Research on redundancy growth and its application in field management showed that appropriate reduction of redundancy growth might result in increased yield (Han et al., 2005).

This experiment of alternate irrigation on wheat/faba bean intercropping glasshouse conditions has also indicated that AI has the good control of the excessive vegetative growth. The ground part growth of intercropped wheat and faba bean with alternate irrigation was much shorter than that of monoculture with conventional irrigation. However, average grain yield per plant in wheat and faba bean plant with alternate irrigation didn't see significant difference.

Furthermore, different irrigation method led to a substantial difference in the root growth pattern and root interactions of component crops in intercropping. The low competitive capacity of legume compared to cereal was ascribed to its small root system and resulting low competitive ability. It is therefore possible that, during a typical drying cycle (about 5 to 7 days), part of the root system near the soil surface comes in contact with dry soil, while other roots continue to extract water from deep and wetter soil layers. Extraction of soil water from deeper layers has been observed in some PRD and surface irrigation studies (Kriedemann and Goodwin, 2003; Canone et al., 2015). These led to a higher faba bean WUE in sole faba bean and wheat/faba bean intercropping with alternate irrigation.

Conclusion

The authors' results have shown that controlled alternate irrigation is better than conventional irrigation. AI showed good physiological responses in P_n, T_r, *G*s, WUE_y and WUE_b, which may provide a useful approach to apply the theory of root-to-shoot long distance signaling process in wheat and faba bean production in areas where irrigation is essential. The extra benefit for sole faba bean and wheat/faba bean intercropping with AI was that the root-shoot ratio was enhanced if compared with CI at the same planting pattern. Furthermore, increasing yield, WUE_y and WUE_b of AI on wheat/faba bean intercropping was also significantly proved in this experiment. It has been suggested that AI had great potential in efficient water use and more data is needed to build in field experiment in arid areas where irrigation is essential and evaporation demand is high.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Ecological diversity and economical importance of species from *Aphanomyces* genus

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Species from *Aphanomyces* genus were investigated in 225 limnologically and trophically different water bodies (springs, rivers, ponds and lakes) as well as in humid soils of north- eastern Poland. Distribution of particular species and their role in diverse ecosystems has been investigated. Thirty taxa, including: 2 species of cultivated parasitic plants, 3 species of parasitic animals, 6 saprotrophic species and 5 species of saprotrophic/ opportunistic species were recorded. Some of *Aphanomyces* species occurred also as parasites of algae, straminipiles and invertebrates. Among parasitic species: *A. cochlioides*, *A. euteiches* (cultivated plant parasite), *A. astaci* (crayfish parasite), *A. piscicida* (fish parasite) playing economically important role were found. Amino acid, carbohydrate and urease assimilation tests were used.

Key words: *Aphanomyces* species, ecological diversity, hydrochemistry, parasite, economical importance.

INTRODUCTION

The number of fish species bred in the control conditions increased in recent years according to Food and Agriculture Organization (FAO, 2012). On the other hand, death on a large scale of the particular fish populations may occur due to bacterial and mycotic infections (Bruno and Wood, 1988). Straminipila from the Saprolegniales order, especially *Saprolegnia*, *Achlya* and *Aphanomyces* species are responsible for eggs infection in the fish species. For example Hatai and Hoshiai (1992) dealt with saprolegniosis of *Oncorhynchus kisutch* demonstrating heavy losses caused by *Saprolegnia parasitica* in the breeding of this species, even up to 50%. *Achlya flagellata* and *A. prolifera* are known to have caused total damage to the incubated eggs of *Tor tor* Lac. in India (Sati and Khulbe, 1981). *Aphanomyces astaci* is known to cause so-called “plague” of crayfish (Schikora, 1903) and *A. laevis* known in mass deaths of rainbow trout during reproduction on the Taiwan (Chien, 1981). Since the mid- 1980s epizootic ulcerative syndrome (EUS) have been described as a disease affecting wild and farmed freshwater and estuarine fish (Chinabut, 1998). It has been reported from Australia, North America, Asia and Africa (OIE, 2007). The agent associated with this disease is the straminipiles organism belonging to *Aphanomyces* genus. In 1997 Kitancharoen and Hatai (1997) described also *A. frigidophilus* as a parasite of the Japan charr eggs.Whereas, such species of the *Aphanomyces* genus as *A. cochlioides* and *A. euteiches* because of their agriculturally importance, are specialized to parasitize roots of sugar beet and Fabaceae species,

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respectively (Bangsund and Leistritz, 1993; Sauvage et al., 2007; Dieguez-Uribeondo et al., 2009).

On account of that, authors have decided to find out which of the already known straminipiles organisms belonging to *Aphanomyces* genus is responsible for the mycotic diseases in plants and animals and can grow in different ecosystems of the north-eastern Poland.

MATERIALS AND METHODS

Description of *Aphanomyces* (de Bary, 1860) genus

Aphanomyces genus was described firstly in 1860 by de Bary (de Bary, 1860) and included initially following four species: *A. laevis*, *A. phycophilus*, *A. scaber* and *A. stellatus*. Other species from this genus have been described by Drechsler (1929) and Scott (1961). At present, according to Index Fungorum, *Aphanomyces* genus comprises 45 taxa and 40 species (David and Kirk, 1997), according to Ballesteros et al. (2006)- 30 species and according to Dieguez- Uribeondo et al. (2009)- 35-40 species. Dick (2001) classifies *Aphanomyces* together with *Leptolegnia* and *Plectospyra* to Leptolegniaceae family. Some species specialize in plant or animal parasitism, other ones are saprotrophic growing on decaying plant and animals debris (Scott, 1961; Fuller and Jaworski, 1987; Dick, 2001; Johnson et al., 2002; Dieguez-Uribeondo et al., 2009). There are also species which are primarily saprotrophic but in some instances become opportunistic pathogens (Royo et al., 2004; Patwardhan et al., 2005). Plant parasitic species include: *A. cochlioides*- pathogen of roots of sugar beet and *P. euteiches* which parasitize on Fabaceae species (Papavizas and Ayers, 1974; Levenfors and Fatehi, 2004). *A. astaci* is a parasite of a freshwater crayfish (Söderhäll and Cerenius, 1999). *A. invadans* and *A. frigidophilus* (*A. piscicida*) devastate both freshwater and estuarine fishes (Chnabut, 1998; Czeczuga et al., 2011a). *A. laevis*, *A. stellatus* and *A. helicoides* belong to opportunistic pathogens (Patwardhan et al., 2005; Dieguez-Uribeondo et al., 2009).

Occurrence environment of *Aphanomyces* species

Material was collected in north- eastern part of Poland within 1985 to 2012. Water samples were collected from 225 limnologically and trophically different water bodies-springs (31), rivers (45), ponds (21) and lakes (128). Oligotrophic, mesotrophic, eutrophic and dystrophic types of lakes were investigated. Water samples were collected at a depth of 10 (spring)- 30cm (other water bodies) and at a distance of 0.5m from the bank, at three sites. Nineteen water parameters were determined (Table 1, only as representatives for particular types of water bodies) according to generally accepted methods (Golterman and Clymo, 1969; APHA, 2005).

The soil species was collected from roots of common bean, common serradella, common vetch, pea and sugar been (Table 4) at farmed land of north-eastern Poland. The amino acid, carbohydrate and urease assimilation tests were performed according to Yuasa and Hatai (1996) and Kitancharoen and Hatai (1998).

Determination of *Aphanomyces* species

Thickness, height and branches of the hyphae were measured in *Aphanomyces* species. The diameter of zoosporangia, zoospores, cystospores, oospores, oogonia and antheridia were defined. Morphological structure of the oogonia, antheridia and their number were also studied. It was investigated, if the oogonia are mono-, diclinous or androgynous. Some parasitic *Aphanomyces* species showed the repeated zoospore emergence (RZE) as adaptation to the parasitic mode of life (Cerenius and Söderhäll, 1985). For parasitic species (plant and animal) general principles of culture procedures were used (Seymour and Fuller, 1987; Roberts et al., 1993; Willoughby and Roberts, 1994; Paterson and Bridge, 1994; Watanabe, 2002). The parasitic species of the sugar beet and of some species of the legumes (soil species) were isolated according to Dyer et al. (2004) and Sauvage et al. (2007) methods. The isolates collected from the necrotic roots of the investigated plants were transferred to the corn meal agar (CMA, Difco, Detroit, MI) supplemented with rifampicin (38 mg/l). For each isolate, a single hyphal tip was transferred to a new CMA plate to insure only one genotype per isolate. The cultures were stored in sterile distilled water and were replaced in storage by culturing on CMA with rifampicin to repeat the process for storage (Parke and Grau, 1992).The taxa of *Aphanomyces* species were identified using the keys of Scott (1961), Batko (1975), Pystina (1994), Johnson et al. (2002) and Petrini and Petrini (2013).

RESULTS

The water samples used for the analysis differed in nutrient content and other parameters (Table 1). The highest nitrogen, sulphates and chlorides content was found in Spring Cypisek, Akcent and Fosa, Komosa Pond. The lowest content of nitrogen, sulphates and chlorides was noted in Hancza Lake. 30 taxa from *Aphanomyces* genus were found in the water bodies and soil of north-eastern Poland (Table 2). Same species occurred in all types of the investigated water reservoirs. *A. astaci*, *A. laevis*, *A. irregularis*, *A. parasiticus* and *A. stellatus* belong to this group. Such species as *A. apophysii*, *A. coniger*, *A. ovidostruens*, *A. polysporus*, *A. scaber* and *A. volgensis* were found only on the plant debris, other species also on animals' substratum (Table 3). *A. cochlioides* and *A. euteiches* are the parasit on cultivated plants (Table 4). 6 *Aphanomyces* species including: *A. frigidophilus*, *A*. *invadans* and *A. piscicida* and others were present on the fish eggs (Table 5). Investigated *Aphanomyces* species assimilated only three amino acids: alanine, glutamine and cysteine and from carbohydrates only glucose and starch, but they did not assimilate urease (Table 6).

DISCUSSION

Thirty taxa *Aphanomyces* were found in the water bodies of north-eastern Poland (Table 2). Five species occurred in all types of investigated water bodies, two species- *A. cochloides* and *A. euteiches* occurred in humid soils. *A. cochlioides* is a parasite of roots of sugar beet, *A. euteiches* of pea and other legumes species roots. The infected roots of plants ranged from 31.8% (common bean) to 50.7% (pea) during humid years. Therefore, soil species of the *Aphanomyces* genus are economically important, especially for the food industry (pea, sugar beet) (Levenfors and Fatehi, 2004) and for some plant

Table 1. Chemical and physical properties of water in represented of particular types of the water bodies (in mg l^{-1}).

Specification	Spring Cypisek	Stream Miahka	River Narew	Pond Komosa	Lake				
					Białe W. (a)	Hańcza (b)	Wigry (c)	Blizno (d)	Suchar (e)
Temperature (°C)	13.5	19.7	16.2	17.4	16.8	16.2	16.4	13.8	19.2
pH	7.4	8.0	8.0	7.6	8.0	8.1	8.2	8.3	5.2
DO	9.0	18.2	15.0	12.8	16.8	21.4	18.8	16.4	7.6
BOD_5	1.8	3.8	3.2	7.4	1.2	1.6	3.4	2.8	2.4
COD	4.6	6.0	7.0	13.2	4.8	5.2	9.5	6.2	27.9
CO_2	11.0	10.4	6.6	8.3	15.4	6.1	10.4	5.3	6.6
Alkalinity in $CaCO_3$ (mval l^{-1})	4.9	3.1	3.5	3.9	1.8	2.4	3.1	3.2	0.4
$N\text{-}NH_3$	0.1	0.05	0.33	0.16	0.05	0.13	0.98	0.27	1.78
$N\text{-}NO_2$	0.01	0.02	0.0	0.01	0.0	0.0	0.01	0.03	0.01
$N\text{-}NO_3$	0.95	0.09	0.0	0.03	0.01	0.02	0.48	0.29	0.03
$P\text{-}PO_4$	0.78	0.48	0.59	0.26	0.08	0.18	0.94	0.15	0.01
Sulphates	56.83	42.3	11.2	42.8	17.4	15.7	10.2	14.1	6.9
Chlorides	31.0	10.5	24.5	23.5	10.2	13.3	18.6	15.2	170.1
Calcium	119.5	64.3	17.2	68.4	23.7	29.7	32.4	45.4	6.5
Magnesium	18.5	10.9	29.1	28.8	6.2	12.1	10.8	12.1	0.01
Iron	0.2	0.52	0.53	1.53	0.0	0.0	0.04	0.12	0.02
Manganese	0.0	0.04	0.03	0.02	0.0	0.01	0.10	0.01	0.0
Dry residue	478.0	286.0	402.0	375.0	86.8	175.4	208.0	196.0	80.0
Dissolved solids	316.0	264.0	256.0	312.0	32.1	161.1	180.0	158.0	8.0
Suspended solids	162.0	22.0	146.0	63.0	14.7	14.3	28.0	38.0	72.0

(a) – oligotrophic; (b) – α-mesotrophic; (c) – β-mesotrophic; (d) – eutrophic; (e) – dystrophic.

Table 2. *Aphanomyces* species founded in particular types of the environment.

Taxa	Cultivated soil	Spring	Stream	River	Pond	Lakes group				
						a	b	c	d	e
A. acinetophagus A. F. Bor. & F.T. Wolf										x
A. americanus (A. F. Bor. & F.T. Wolf) W.W. Scot									x	
A. *amphigynus* Cutter		x		x	x			x	x	
A. *apophysii* Lacy			x		x			x		
A. *astaci* Schikora		x	x	x	x	x	x	x	x	x
A. bosminae W.W. Scott		x			x				x	
A. cochlioides Drechsler	x									

Table 2. (Contd.)

A. coniger H.E. Petersen			x		x			x		
A. daphniae Prowse		x		x	x				x	
A. euteiches Drechsler	x									
A. eut. f. sp. *phaseoli* W.F. Pfen. & D.J. Hag.	x									
A. eut. f. sp. *pisi* W.F. Pfen. & D.J. Hag.	x									
A. exoparasiticus Coker et Couch			x		x		x			
A. frigidophilus Kitanch. & Hatai		x	x	x	x				x	
A. helcoides Minden		x	x							
A. hydatinae Valkanov								x		
A. invadans Willoug. et al.					x					
A. irregularis W.W. Scott		x	x	x	x	x	x	x	x	x
A. keratinophilus (Okub. & Kob.) R.L. Seym. T.W. Joh.		x		x					x	
A. laevis de Barry		x	x	x	x	x	x	x	x	x
A. norvegicus Ville		x	x							
A. ovidestruens Gickelh.				x					x	
A. parasiticus Coker		x	x	x	x	x	x	x	x	x
A. phycophilus de Bary			x		x					
A. piscicida Hatai				x	x					
A. polysporus Milovtz.					x					
A. scaber de Barry									x	
A. sparrowii Cutter									x	
A. stellatus de Bary		x	x	x	x	x	x	x	x	x
A. volgensis Domash.			x	x						
Total number of taxa	4	12	13	12	16	5	6	9	14	6

species used as animal foodstuff for domestic animals (Papavizas and Ayers, 1974; Holub et al., 1991; Brantner and Windels, 2001).

Most *Aphanomyces* species were found in such eutrophic reservoirs of the stagnant water bodies as ponds (16) and eutrophic lakes (14 species), (Table 2). Only a few *Aphanomyces* species were found in water with a small content of the biogenic substances. There are oligotrophic, α-mesotrophic and dystrophic lakes (5-6 species). In the flowing waters (springs, streams, rivers) the authors found many (12-13) *Aphanomyces* species and such species as *A. astaci*, *A. irregularis*, *A. laevis*, *A. parasiticus* and *A. stellatus* were found in all types of the investigated water bodies. According to this fact they are called the eurytrophic species and have a wide range of the ecological tolerance. There are various organic compounds of carbon, free saccharides, free amino acids, numerous enzymes and other compounds (Hoagland et al., 1993) called extracellular products (being excreted by the phytoplankton and makrophytes) which exert the influence on the mycotal species in water. They are a medium for different heterotrophic organisms including mycotal species. As it is known, in oligotrophic, α-mesotrophic and dystrophic lakes there is a little amount of biogenic compounds, the phytoplankton and the macrophytes excreting less extracellular products serving as nutrients for heterotrophic organisms.Such species as *A. apophyscii*, *A. coniger*, *A. ovidostruens*, *A. polysporus*, *A. scaber* and *A. volgensis* were

Table 3. Ecological-physiological groups of the *Aphanomyces* species founded in north-eastern Poland.

Farming plants parasite	Algal-fungal parasite	Crayfish, fish parasite	Invertebrate parasite	Saprotrophs species	Saprotroph/opportunis species
A. cochlioides	*A. apophysii*	*A. astaci*	*A. acinetophagus*	*A. amphigynus*	*A. frigidophilus*
A. euteiches	*A. exoparasiticus*	*A. invadans*	*A. americanus*	*A. coniger*	*A. irregularis*
A. eut. f. sp. *phaseoli*	*A. norvegicus*	*A. piscicida*	*A. bosminae*	*A. helicoides*	*A. laevis*
	A. phycophilus		*A. daphniae*	*A. keratinophilus*	*A. parasiticus*
A. eut. f. sp. *pisi*	*A. scaber*		*A. hydatinae*	*A. polysporus*	*A. stellatus*
	A. sparrowii		*A. ovidestruens*	*A. volgensis*	
Number 4	6	3	6	6	5

Table 4. The infection of some farming plants by two *Aphanomyces* species.

Cultivated plant	*Aphanomyces* species	% infected plants	
		Normal[1] season	Humidity[2] season
Common bean (*Phaseolus vulgaris* L.)	*A. euteiches* f. sp. *phaseoli*	11.6	31.8
Common serradella (*Ornithopus sativus* L.)	*A. euteiches*	26.1	38.4
Common vetch (*Vicia sativa* L.)	*A. euteiches*	19.8	49.2
Pea (*Pisum sativum* L.)	*A. euteiches* f. sp. *pisi*	24.2	50.7
Sugar beet (*Beta vulgaris* L.)	*A. cochlioides*	18.4	42.5

1. 4-6 clouded and rainy day in one month (P – 18 mm/month); 2. 12-18 clouded and rainy day in one month (P – 174 mm/month).

Table 5. The infection of crayfish and fish by some *Aphanomyces* species.

Aphanomyces **species**	**Infection**	**Authors**
A. astaci	-Plague of *Astacus* species and other crayfish	Söderhäll and Cerenius (1999)
A. frigidophilus	- 22.8% eggs of Atlantic salmon (*Salmo salar* L.) - Mass mortality of wild and farmed fish	Czeczuga et al. (2011°) Lilley et al. (2003)
A. invadans	- 3.6% of eggs of Chinook salmon (*Oncorhynchus tschawytscha* Wal.)	Czeczuga et al. (2011b)
A. irregularis	- Mass mortality of rainbow trout (*Oncorhynchus mykiss* Wal.)	Chien (1981)
A. laevis	- 11.4% eggs of Chinook salmon (*Oncorhynchus tschawytscha* Wal.)	Czeczuga et al. (2011b)
A. parasiticus	- 2.5% of eggs of Atlantic salmon (*Salmo salar* L.)	Czeczuga et al. (2011a)

Table 6. Amino acid, carbohydrate and urease assimilated by two *Aphanomyces* species.

Species	Amino acid	Carbohydrate	Urease
A. *frigidophilus*	Ala, Cys, Glu	Glu Sta, Tre	-
A. *laevis*	Glu	Glu, Sta	-

Abbreviations: Amino acids: Ala – alanine; Cys – cysteine; Glu – glutamine. Carbohydrate: Glu – glucose; Sta – starch; Tre – trehalose.

found only on the plant substratum, other species also on the animal substratum. The 9 *Aphanomyces* species present in fish, *A. invadans* and *A. piscicida* occurred only on fish eggs. *A. euteiches* is the parasite of Fabaceae family (Papavizas and Ayers, 1974). *A. astaci*, *A. invadans* and *A. piscicida* are animal parasites. *A. astaci*, which has been described by Schikora (1903), infects freshwater crayfish (Söderhäll and Cerenius, 1999). Both *A. invadans* and *A. piscicida* devastated natural and cultured stocks of freshwater and estuarine fish (Chinabut, 1998; Johnson et al., 2004). Some authors confirmed this both species as synonym (Dieguez-Uribeondo et al., 2009), others- as separate species (Phadee et al., 2004a, b). *A. piscicida* was described by Hatai (1980) and causes mycotic granulomatosis in fish (MG) (Egusa and Masuda, 1971), whereas *A. invadans* was described by Willoughby et al. (1995) and is an agent of EUS (Epizootic ulcerative syndrome). Kitancharoen and Hatai (1997) described new species of *A. frigidophilus* from eggs of Japan charr. In Europe, first *A. frigidophilus* was described on eggs of some species of coregonide fishes (Czeczuga et al., 2004). Two years later, Ballesteros et al. (2006) found this species in cuticule of dead crayfish *Austropotamobius pallipes* in Spain and Kiziewicz et al. (2013) observed its occurrence in water from the some springs of north- eastern Poland. Growth of *A. frigidophilus* was also stated on eggs of some species from *Salmo* genus (Czeczuga et al., 2004, 2011a), sturgeonids, Chinook salmon (Czeczuga et al., 2011b) and African catfish (Czeczuga et al., 2013).

As shown in Table 3, such species as: *A. irregularis*, *A. laevis*, *A. parasiticus* and *A. stellatus* were found on plant debris and on fish eggs. They all belong to opportunistic pathogens, which are sapro- and necrotrophic species similar to many species belonging to *Achlya* and *Saprolegnia* genera. Accourding to our study such saprotrophic species as *A. amphiginus* and *A. helicoides* were growing on decaging invertebrate animals and plant debris. According to Dieguez-Uribeondo et al. (2009). *A. helicoides* belongs to saprotrophic- opportunistic group. The most numerous pathogens from this group on fish eggs are: *A. laevis* (41 species) and *A. stellatus* (29). *A. irregularis* was found on eggs of 8 fish species, *A. parasiticus*- on 7. Mentioned species were also present on the eggs of lamprey (Czeczuga, 1997). According to Batko (1975) and Pystina (1994) *A. irregularis* is saprotrophic species and *A. parasiticus*-parasite on other fungal species, especially from *Achlya* genus. *A. laevis* both- eggs and adult individuals of many economically valuable fish species (Lartzeva, 1986; Dudka et al., 1989). *A. bosminae* and *A. daphniae* (occurring on fish eggs) were found in some of investigated water bodies. *A. bosminae* was firstly described by Scott (1961) and first investigated fungus in America. It is a parasite of freshwater crustacean from the *Bosmina* genus. The authors found it on the eggs of *Leuciscus leuciscus* in water from River Suprasl (Czeczuga and Muszynska, 1999). *A. daphniae* is known to be a parasite of the plankton crustacean *Daphnia hyalina* (Leydig) (Prowse, 1954). In our study its growth was revealed on the eggs of *Cobitis aurata* (Filippi) in River Suprasl water (Czeczuga and Muszynska, 1997).

The occurrence of *Aphanomyces* species has been registered in water bodies of African continent by El- Sharouny and Badram (1985), Shaheen et al. (1999), El- Hissy et al. (2004) and Ali (2009). *Aphanomyces* species have been associated with skin lesions and mortality of some fish species. This phenomenon has been observed in two species of fishes from excavated earthen pond at the western shore of the Suez Canal, Egypt, during the winter of 1971 (Shaheen et al., 1999). According to Lilley et al. (2003) this invasive *Aphanomyces* fish infection has been reported by Shaheen et al. (1999) and has been caused by *A. invadans* species. It has also been observed on skin-muscles on aquarium species of *Labeo bicolour* Smith specimens and on the alevins of the Nile tilapia- *Oerochromis niloticus* L.

The assimilation of the amino acids, carbohydrate and urease by two investigated *Aphanomyces* species was very small. Specimens of *A. frigidophilus* from fish eggs have assimilated only 3 amino acids, 3 carbohydrates, whereas *A. laevis* assimilated only one amino acid and 2 carbohydrates. Whereas specimens of *Saprolegnia*, *Achlya* or *Pythium* assimilated 4 to 5 amino acids, 9 to 16 carbohydrates and urease (Kitancharoen and Hatai, 1998). The specimens of both species from *Aphanomyces* genus did not assimilate urease.

Conclusions

All together, 30 taxa of *Aphanomyces* genus occurred in 225 water bodies and some farming land of north-eastern Poland. This straminipiles species was found in limnologically and trophically different springs, rivers, ponds and lakes. Two species were found in farming land. All 30 *Aphanomyces* taxa belonged to four groups: a) plant parasite species, b) animal parasites, c) saprotrophic/opportunistic species and d) saprotrophic species. Group a) consists of: *A. cochlioides* and *A. euteiches*, group b) *A. astaci*, *A. invadans* and *A. piscicida*. *A. astaci* are etiologically an agent of the crayfish plague. *A. invadans* and *A. piscicida* infect freshwater and estuarine fishes as well as devastation of natural and cultured stocks (epizootic ulcerative syndrome). Some species, for example: *A. acinetophagus*, *A. exoparasiticus*, *A. hydatinae* and *A. sparrowii* occurred on some algae, straminipiles and invertebrates species as their parasites. Group c) - *A. frigidophilus*, *A. irregularis*, *A. laevis*, *A. parasiticus* and *A. stellatus* belong to the group of saprotrophic/opportunistic. These species are growing on decaying plant and animal debris (saprotrophic) and on fish eggs (parasitic). Fourth group- d) includes saprotrophic species fragments which grow only on decaying plant and animal debrits. Representatives of this group are: *A. amphiginus, A. coniger*, *A. helicoides*, *A. keratinophilus*, *A. polysporus*, and *A. volgensis*. Pathogenic *Aphanomyces* species in soil and in water bodies play economically significant role.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

A comparative study on nitrogen response among Upland, IRHTN, DRR and other released rice groups

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A field experiment was conducted to investigate the effect of nitrogen (N) fertilizer on growth, development and yield of Upland, IRHTN, DRR and other released rice groups. Six varieties from each group, a total of 18 genotypes were planted in randomized complete block design during dry (2011) and wet (2012) seasons with three replications at Indian Institute of Rice Research, Hyderabad. Group responses to the N-stress (N0; native nitrogen) and recommended nitrogen (N100; 100 kg N ha^{-1}) for physiological, morphological and yield attributes were recorded. The average leaf rolling time was found 75.4% increased with N-100 in IRHTN group over N-0 in the same group and also found higher among the groups. Leaf temperature, SPAD was noticed higher in DRR and other released group with N-100. The highest plant height was observed in Upland group only, while number of tillers, effective booting tillers (EBT), filled grain weight and total dry matter (TDM), harvest index (HI), total nitrogen content (straw + grain) were found increased with N-100 in DRR and other released group.

Key words: Leaf rolling, nitrogen, rice, SPAD, grain yield.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the important cereal crops, grown in a wide range of agro climatic zones, to afford food for half of the world's increasing population. It is cultivated on about one-tenth of the earth's arable land (Naheed et al., 2007). In sustainable and climate smart rice cultivation, adequate usage of nitrogen fertilizer thereby minimizing risk to environment, optimizing grain yield and lowering production cost have been the key objectives since the beginning of the twentieth century (Koutroubas and Ntanos, 2003). Nitrogen being a macronutrient takes part in central role of determining grain quality of cereal crops. It is a yield-limiting nutrient in irrigated rice production around the world (Ladha and Reddy, 2003; Samonte et al., 2006). Soil organic N and N derived from biological nitrogen fixation by associated organisms are major sources of N for lowland. Soil organic N is incessantly lost through plant removal, leaching, de-nitrification and ammonia volatization. An additional concern is that the capacity of soil to supply N may diminish with continuous demanding rice cropping

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under wetland condition. More than 50% of the N fertilizer used by flooded rice is derived from the combination of soil organic N and biological nitrogen fixation by free-living and rice plant-associated micro biota. The remaining N requirement is normally met with fertilizer (Motior Rahman et al., 2009). Rice plants require N in all growth stages of its life cycle, during vegetative stage to promote growth and tillering, which in turn determines potential number of panicles. During early panicle initiation stage nitrogen contributes to spikelet production and to sink size during the late panicle formation stage. It also plays an important role in grain filling, improving the photosynthetic capacity, and promoting carbohydrate accumulation in culms and leaf sheaths (Mae, 1997). Earlier studies revealed that judicious and proper use of fertilizers markedly increase the yield and improve the quality of rice (Place et al., 1970).

Besides giving importance to N fertilizer usage, it is essential to find out the suitable N dosage for a rice group and its performance under native nitrogen, as the excessive application of fertilizers may not necessarily increase the yield (Samonte et al., 2006). A recent research revealed that in aromatic rice group Basmati 370 and Ranbir basmati were performed well under 'N' stress conditions by Vijayalakshmi et al. (2015). A recent research on aromatic rice revealed that Basmati 370 and Ranbir basmati were performed well under 'N' stress conditions (Vijayalakshmi et al., 2015).

In view of the above fact that all the rice groups do not require the same N dosage. Screening of Upland, IRHTN, DRR and other Released groups under N-0 and N-100 levels was investigated for features of their physiological and yield adaptability.

MATERIALS AND METHODS

A field experiment was conducted with the objective to evaluate eighteen genotypes belongs to three groups [Upland (IR 82635-B-B-82-2, IR 82590-B-B-98-2, IR 82635-B-B-25-4, CT 15671-15-4-2-2-2-M, CT 15696-3-3-5-1-1-M, PR 26703-3B-PJ 25), IRHTN (GIZA 176, ZARDROME (ACC 32379), SAKHA 104, IR 22, IDSA 77, IR 60), DRR and other Released (Improved samba masuri, Nagarjuna, Mandya Vijaya, Swarna, Anjali, Pooja)] for association of physiological traits to identify nitrogen use efficiency. A stress treatment was given to rice varieties as low N (N0, native nitrogen) and recommended nitrogen (N100, 100 kg N ha^{-1}).

Observations

In situ leaf chlorophyll content (Minolta Corporation's Chlorophyll SPAD-502 plus USA), and leaf temperature using IR thermometer (Fischer Scientific USA) were measured. Leaf rolling was determined *in vivo* between 11.00 AM -12.30 Noon as described in DRR annual report (2007-2008). Nitrogen content from straw and grain was estimated according to the Kjeldahl method of N estimation (1883). At the physiological maturity phase, morphological characters like plant height, effective booting tillers were recorded. Yield components were recorded from five hills. Data of the two seasons is pooled and analyzed using statistical program Statistix 8.1 (Analytical Software Inc. USA).

RESULTS AND DISCUSSION

Highest leaf rolling time was recorded in IRHTN group and not significantly different to Upland with N-100 treatment (Figure 1). In upland group the leaf area reduction was noticed significantly less under N-0 among the groups (Figure 2). Leaf rolling in most of the cereals is a dehydration avoidance mechanism and protects the leaves from photo damage due to high light intensity (Corlett et al., 1994; Kadioglu and Terzi, 2007). Leaf rolling was performed by specialized cells of the leaf known as bulliform cells, movement of which results in rolling of leaf. Turner et al. (1986) reported that sufficient decrease in water potentials induce leaf rolling in order to reduce water loss. The bulliform cell enlargement within the mesophyll layer is a logical response to higher N since the function of bulliform cells is to control leaf movements (Buleon et al., 1998) and more turgid and larger leaves were observed in plants having higher N levels, that shows more leaf area exposure for sunlight leads to better photosynthesis ultimately gives more yield over N-0. The variety which maintains the turgid condition for long time is known as tolerant. In the study IRHTN group was noticed as it has taken longer duration for leaf rolling under recommended N level, but the interesting point is under N-0 upland group recorded longer duration for leaf rolling.

Marginal increase was found in leaf temperature with nitrogen application over low nitrogen leaves (Figure 3). Nitrogen application increased leaf temperature by 30.9% in DRR and other released group, minimum increase for N-application in upland group 6%. In general photosynthesis at normal CO_2 is relatively limited by Rubisco capacity under high-temperature conditions, but in the present study it shows a marginal increase with nitrogen application leads to increase the rate of photosynthesis.

A considerable reduction in SPAD values were recorded under N-stress conditions (Figure 4) in all the groups. Amongst, nitrogen application increased SPAD value by 41.8% in DRR and other released group and minimum increase was found in upland group by nitrogen application (17.6%). The higher rate of nitrogen application at higher SPAD value was conducive for tillers production. This might be due to favourable effect of nitrogen on cell division and tissue organization that ultimately improved tillers formation at tillering growth stage (Huang et al., 2008).

Group wise responses for the application of nitrogen to morphological components are presented in Table 1. Amongst, upland rice group recorded highest plant height with nitrogen application and there is no significant difference between the other groups in plant height under N-100. But the treatment difference was found highest in IRHTN group (37.3%). The other two groups (Upland 26.5%, DRR and other released group 26.7%) were not significantly different. Number of tillers and productive tillers were recorded highest in DRR and other released

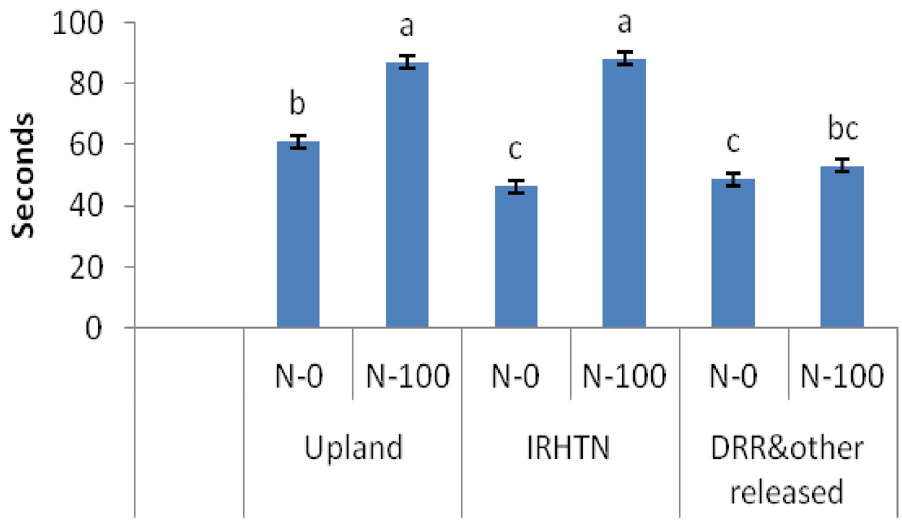

Figure 1. Leaf rolling time in both nitrogen conditions from three groups.The data presented are means and their standard errors (*n*= 5). Means followed by the same letters in column are not significantly different at P= 0.05.

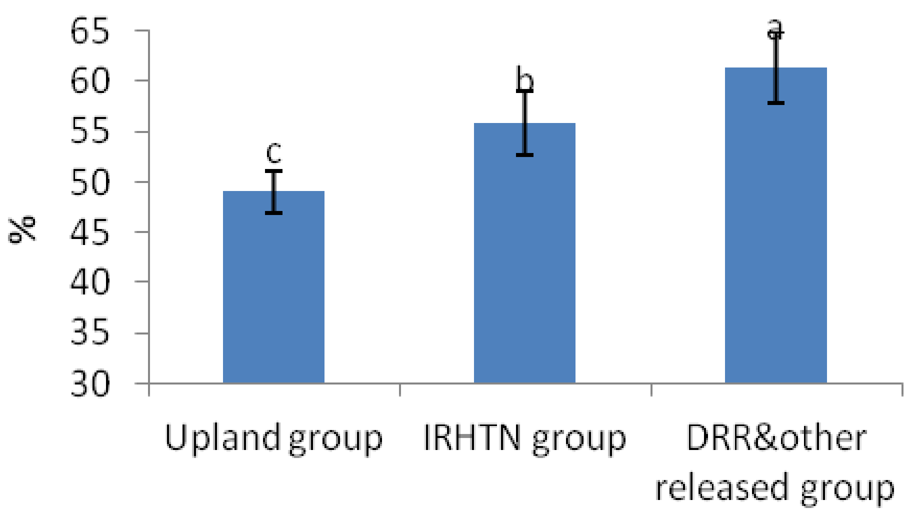

Figure 2. Leaf area reduction in three rice groups under nitrogen stress. The data presented are means and their standard errors (n= 5). Means followed by the same letters in column are not significantly different at P= 0.05.

group in N-100 field.

Amongst, DRR and other released group panicle weight, filled grain weight, total grain weight, TDM and HI were found higher with nitrogen application (Table 2). Nitrogen fertilizer is a major essential plant nutrient and key input for in increasing crop yield (Dastan et al., 2012). Nitrogen contributes to carbohydrates accumulation in culms and leaf sheaths during the pre-heading stage and in the grain during the ripening stage of rice (Swain et al., 2010). Amongst, DRR and other released group were found significantly higher in total (Straw and grain) nitrogen content in N-100 (Table 3). In N-0 the grain weight was found significantly higher in upland group than the other groups. The same trend was following from the physiological aspects (leaf rolling) to yield attributes (filled grain weight).

Conclusion

In the present study, N-100 proved significant increase in growth and yield in all the groups. Amongst, DRR and

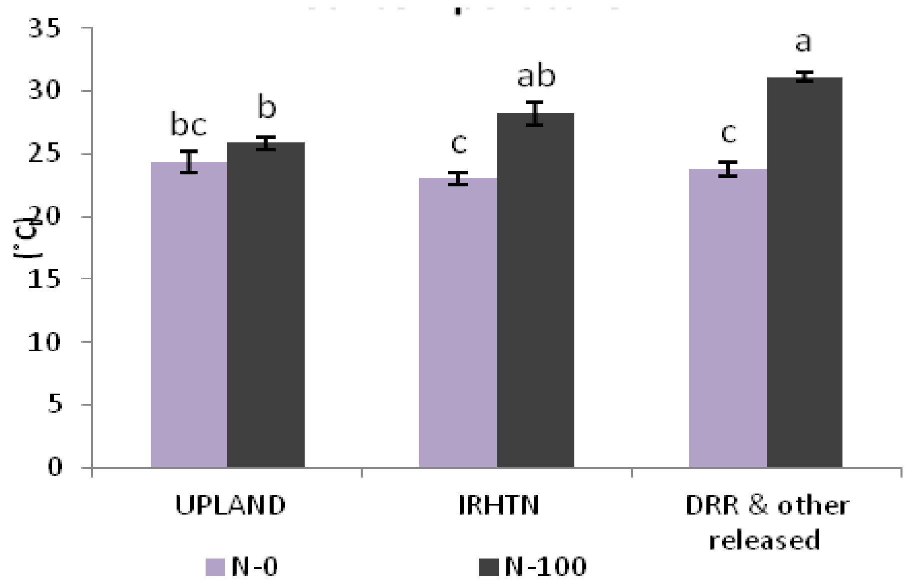

Figure 3. Leaf temperature influenced by nitrogen application in three rice groups. The data presented are means and their standard errors (n= 5). Means followed by the same letters in column are not significantly different at P= 0.05.

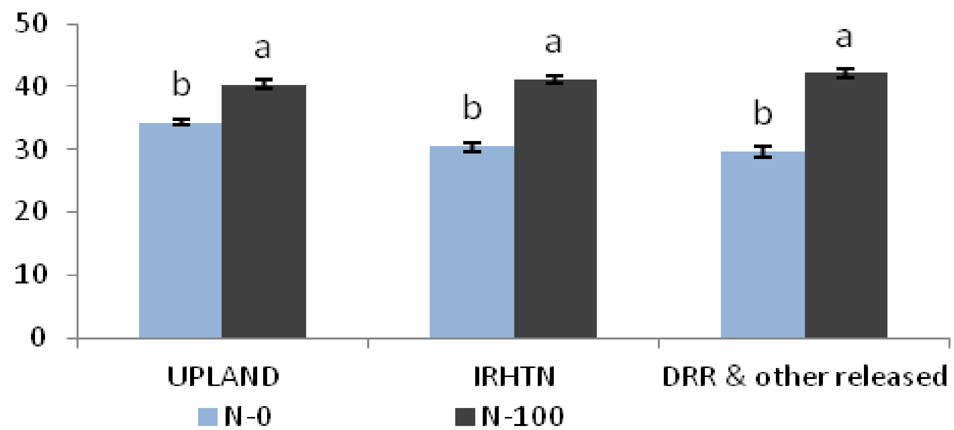

Figure 4. Influence of nitrogen on SPAD value in three rice groups. The data presented are means and their standard errors (n= 5). Means followed by the same letters in column are not significantly different at P= 0.05.

Table 1. Morphological changes as influenced by N application in three groups of rice.

Group	Treatment	Plant height (cm)	Tillers	EBT
Upland	N0	79.02 ± 0.3 ^{b}	5.38 ± 0.4 ^{b}	4.97 ± 0.2 ^{c}
	N100	100.13 ± 0.7 ^{a}	8.44 ± 0.2 ^{a}	8.36 ± 0.5 ^{a}
IRHTN	N0	67.83 ± 0.3 ^{b}	6.77 ± 0.1 ^{b}	5.97 ± 0.3 ^{bc}
	N100	92.27 ± 0.11 ^{a}	8.77 ± 0.5 ^{a}	7.50 ± 0.6 ^{b}
DRR & other released	N0	71.05 ± 0.8 ^{b}	8.05 ± 0.3 ^{a}	7.44 ± 0.2 ^{b}
	N100	90.38 ± 0.10 ^{a}	9.33 ± 0.7 ^{a}	9.11 ± 0.8 ^{a}

The data presented are means and their standard errors (n= 5). Means followed by the same letters in column are not significantly different at P=0.05.

Table 2. Influence of nitrogen yield and its components in three rice groups.

Group	Treatment	Panicle weight/hill (g)	Filled grain weight/hill (g)	Total grain weight/hill (g)	TDM (g)	HI%
Upland	N0	8.55 ± 0.6 [b]	7.34 ± 0.3 [b]	7.81 ± 0.4 [b]	16.91 ± 0.12 [b]	42.14 ± 0.22 [b]
	N100	13.3 ± 0.9 [a]	11.34 ± 0.7 [a]	12.13 ± 0.7 [a]	25.95 ± 0.16 [a]	44.30 ± 0.13 [a]
IRHTN	N0	6.77 ± 0.4 [c]	5.88 ± 0.6 [c]	6.21 ± 0.3 [bc]	12.55 ± 0.11 [c]	42.59 ± 0.18 [b]
	N100	8.77 ± 0.3 [b]	7.40 ± 0.4 [b]	7.96 ± 0.8 [b]	16.58 ± 0.14 [b]	44.19 ± 0.15 [a]
DRR & other released	N0	5.85 ± 0.6 [d]	4.95 ± 0.5 [cd]	5.32 ± 0.2 [c]	12.02 ± 0.10 [c]	42.23 ± 0.13 [b]
	N100	14.21 ± 0.7 [a]	12.29 ± 0.9 [a]	13.09 ± 0.11 [a]	27.22 ± 0.13 [a]	44.75 ± 0.17 [a]

The data presented are means and their standard errors (n=5). Means followed by the same letters in column are not significantly different at P=0.05.

Table 3. Total nitrogen content in three rice groups.

Group	Treatment	Grain (%)	Straw (%)	Total N content
Upland	N0	1.02 ± 0.8 [b]	0.38 ± 0.11 [bc]	1.40 ± 0.19 [c]
	N100	1.13 ± 0.5 [a]	0.40 ± 0.18 [b]	1.53 ± 0.23 [b]
IRHTN	N0	0.99 ± 0.3 [bc]	0.40 ± 0.14 [b]	1.39 ± 0.17 [c]
	N100	1.04 ± 0.7 [b]	0.42 ± 0.11 [b]	1.44 ± 0.18 [c]
DRR & other released	N0	1.06 ± 0.6 [b]	0.42 ± 0.19 [b]	1.48 ± 0.25 [bc]
	N100	1.14 ± 0.5 [a]	0.48 ± 0.12 [a]	1.62 ± 0.17 [a]

The data presented are means and their standard errors (n= 5). Means followed by the same letters in column are not significantly different at P= 0.05.

other released group were performed well in N-100, under N stress conditions poor performance noticed in same group. But Upland group has shown better performance among the three groups under N-0. Thus it is concluded that, under nitrogen stress conditions upland group shows better performance and with recommended nitrogen fertilizer DRR & other released group gives better yield.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Effect of *Annona muricata* L. (1753) (Annonaceae) seeds extracts on *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae)

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The use of botanical acaricides extracted from plants as an alternative to replace the chemical acaricides is an interesting and efficient option to control pests and ameliorate their toxic effects to humans and the environment. The aim of this work was to evaluate the effect of seed extracts of *Annona muricata* (Annonaceae) to control the mite *Tetranychus urticae* (Acari: Tetranychidae) using disks of 5.0 cm in diameter jack bean leaves, *Canavalia ensiformis* (Fabaceae) as a substrate. The ethanolic extract of the seeds showed the highest toxicity to the mite, with LC_{50} around 1.77 mg/ml, followed by hexanic and aqueous extracts, with LC_{50} estimated at 3.29 and 151.74 mg/ml, respectively. Abamectin caused mortality of 40% to *T. urticae* in a commercial dosage of 100 ml/100 L. The repellent effect of the ethanolic extract, the toxicity on eggs and the residual effect on mites were also evaluated. The concentrations of 0.61, 0.88 and 1.77 mg/ml, as well as Abamectin had neutral effects on *T. urticae* and the concentrations of 3.10, 5.11 and 12.07 mg/ml were repellent. The viability of the eggs when sprayed with the ethanolic extract (LC_{99}), Abamectin and the control was 9.5, 76.5 and 91.5%, respectively. The residual effect of ethanolic extract was 120 h after application (HAA), with mortality rates above 80%; Abamectin presented residual effect of 48 HAA with 33.3% mortality. In this way, the ethanolic extract of *A. muricata* proved to be a promising product to the control of *T. urticae*.

Key words: Botanical acaricide, spotted spider mite, soursop.

INTRODUCTION

The spider mite, *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae), is one of the most important pests worldwide causing considerable losses in several economically important crops such as cotton, apple, vine, bean, strawberry, papaya, potatoes, tomatoes and other vegetables, ornamental and medicinal plants (Miresmailli and Isman, 2006; Moraes and Flechtmann, 2008).

Its importance as a pest is related to its high ability to

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develop resistance to chemical acaricides, its polyphagous habit, high breeding potential and short biological cycle. High infestations can cause discoloration of leaves, loss of photosynthetic capacity and, eventually, death of plants (Devine et al., 2001).

Chemical control with acaricides is still the most widely used method, but in many cases inefficiently, as its intensive use has selected resistant populations of mites (Sato et al., 2007), in addition, can cause upwelling of pests due to the mortality of natural enemies (Ferla and Moraes, 2006; Marsaro Júnior et al., 2012).

These negative effects of chemical acaricides could be mitigated by the use of botanical product extracted from plants with acaricide power. Their peculiar characteristics relating to efficiency, rapid degradation and low impact on natural enemies, humans and the environment may bring considerable advantages on their commercial use (Brito et al., 2008), as can be found in *Annona muricata* (soursop) seeds.

Forty-two species of Annonaceae family, distributed in 14 genera with emphasis on *A. muricata* and *Annona squamosa* L., with potential for insecticide/acaricide have already been reported (Oliveira and Pereira, 2009; Krinski et al., 2014).

The acetogenins present in these plants are important secondary metabolites that are responsible to the bioactivity of several species of Annonaceae (Alali et al., 1999), being found in the leaves, twigs, roots and seeds (Castillo-Sanchez et al., 2010). The acetogenins act as inhibiting mitocondrial electron transport and so affecting the NADH-ubiquinone oxidoreductase action (Álvarez et al., 2007).

The present work had the objectives to evaluate the lethal toxicity, repellent effect, egg toxicity and residual effect of seed extracts of *A. muricata* to control the spider mite *T. urticae*.

MATERIALS AND METHODS

The experiments were carried out at the Laboratory of Entomology and in the greenhouse of the Centrode Ciências Agrárias of the Universidade Federal de Alagoas (UFAL) in Rio Largo, Brazil.

Collecting and rearing spotted spider mite *T. urticae*

The mite was collected on infested rose bouquets from flower shops in Maceio, Alagoas. For identification and confirmation of species *T. urticae*, mounting was accomplished of specimen in lamina for microscopic and mayo of Hoyer. The identification was facilitated from the Entomology and Acarology Laboratory of UFAL *Campus* of Arapiraca. The mites were reared on plants of jack bean, (*Canavalia ensifomis* L. DC) (Fabaceae) inside cages of 0.50 × 0.50 × 0.50 m covered by fine mesh fabric, under room temperature of 26 ± 2°C, relative humidity 60 ± 10% and photophase of 12 h. This procedure was done to obtain pest population under the laboratory.

Obtaining seeds and preparation of the extracts

A. muricata seeds were washed in tap water and placed on paper towels to remove excess of water. The seeds were then placed in paper bags and dried out in an oven with air circulation at the temperature of 50°C for seven days. After that the seeds were grounded on a knife mill type Wiley to obtain a fine powder.

The extracts were prepared in the Laboratory of Natural Resources at UFAL. For the preparation of organic extracts, 6 kg of seeds powder was used, submitted to cold extraction in stainless steel percolator tube, first with hexane CH_3 (CH_2) $4CH_3$ (EH) by 24 h, then on the resulting pie was added ethanol (CH_3CH_2OH) (EE) for three times during 72 h. The solutions were filtered and concentrated in a rotary evaporator at 50ºC at reduced pressure. The aqueous extract H_2O (EA), was obtained from the seeds powder submitted to extraction in water at 35% (m / v) in stainless steel percolator tube for 48 h.

Toxicity of extracts

Pretesting were undertaken using different concentrations of the extracts to determine the upper limit values (100% mortality) and the lower limit (no mortality). The extracts were diluted in distilled water with Tween 80 (0.05%), at the following concentrations: for the aqueous extract (EA) 111.11; 176.47; 250.0; 333.33; 428.0; 538.46 mg/ml; for the hexanic extract (EH) 1.69; 3.39; 5.10; 6.81; 8.53; mg/ml 10.26 and for the ethanolic extract (EE): 0.88; 1.76; 3.53; 5.31; 7.10; 8.88 mg/ml. A control solution of distilled water and Tween 80 (0.05%) was used. The extracts were also compared with the application of the acaricide Abamectin as a positive control (Abamectin Nortox® EC 18 gl; Zhejiang Hisun Pharmaceutical Company Ltd) in a commercial dosage (100 ml/100 L).

Foliar disks of 5.0 cm in diameter of jack bean leaves were sprayed with the different treatments using a Potter Tower (Burkard, Rickmansworth, UK). The procedure was performed at a pressure of 5 psi/pol² in a volume of 2.3 ml extract, corresponding to a deposit of 1.9 ± 0.37 mg/cm², in accordance with the recommendation of the IOBC/WPRS (Reis et al., 1998).

The disks were then placed to dry out on paper towels at room temperature for an hour and put to float on water, inside Petri dishes (8.5 cm diameter), as the methodology described by Reis and Alves (1997). A total of 10 females of *T. urticae* were transferred to each disc, with the aid of thin brush bristles, totaling six replicates per treatment.

The mortality was evaluated 72 h after application (Sato et al., 2002) and lethal concentrations (LCs) estimated by the Probit analysis in the statistical program SAS (SAS Institute, 2002).

Repellent effect

For the repellency test EE and Abamectin was used. Disks (5.0 cm in diameter) were immersed for five seconds at each treatment or control (Tween 80 distilled water), placed side by side, and connected by a glass cover slip (18 × 18 mm). This set was placed on filter paper saturated with distilled water inside Petri dishes (14 cm in diameter), according to methodology adapted from Esteves Filho et al. (2010).

The tested concentrations of the EE were obtained on concentration-response curve, equivalent as LCs 10, 20, 50, 75, 90 and 99 and the Abamectin (100 ml/100 L). In the center of the cover slip 10 females of *T. urticae* were released, and after 2 h assessed the number of females in each disk, being 15 repetitions per treatment.

The repellency index (RI) was calculated by the formula: RI=2G/(G+P) according to Kogan and Goeden (1970), where G is

Table 1. Lethal concentration (LC) of the seed extracts of *Annona muricata* on females of *Tetranychus urticae*

Treatment	n[a]	DF[b]	Inclination ± SE	LC_{50} (mg/ml) (CI 95%)	LC_{99} (mg/ml) (CI 95%)	χ^{2c}	P
Aqueous extract	360	4	2.94 ± 0.35	151.74 (126.36-173.65)	933.38 (698.71-1472.0)	7.6	0.10
Hexanic extract	360	4	2.36 ± 0.43	3.29 (1.91-4.45)	31.87 (16.01-236.78)	9.2	0.06
Ethanolic extract	360	4	2.80 ± 0.26	1.77 (1.50-2.05)	12.07 (9.22-17.57)	7.7	0.10

[a]Number of mites used in each experiment; [b]Degree of freedom of Chi-square; [c]Chi-square.

Table 2. Repellent effect of the ethanolic extract (EE) of *Annona muricata* seeds on females of *Tetranychus urticae*.

Treatment	Concentration	Mean of repellency index[a] (±SD[b])	Effect
Abamectin (ml/L)	100 /100	1.18 ± 0.40	Neutral
	0.61 (LC_{10})	0.74 ± 0.27	Neutral
	0.88 (LC_{20})	0.74 ± 0.27	Neutral
Ethanolic extract (mg/ml)	1.77 (LC_{50})	0.86 ± 0.38	Neutral
	3.10 (LC_{75})	0.41 ± 0.19	Repellent
	5.11 (LC_{90})	0.32 ± 0.38	Repellent
	12.07 (LC_{99})	0.16 ± 0.22	Repellent

[a]Repellency index calculated according to Kogan and Goeden (1970). [b]SD: Standard deviation.

the number of mites in the treatment and P is the number of mites in control. The used safety interval period used to consider whether or not the treatment is repellent was obtained from the average of the calculated RI and its standard deviation (SD). If the average of RI was less than 1 - SD, the extract was repellent. If the average of RI was greater than 1 + SD, the extract was attractive; and if the average was between 1- SD and 1 + SD the extract was considered as neutral.

Toxicity on eggs

Females of *T. urticae* were transferred to disks (3.0 cm in diameter) inside Petri dishes (14.0 cm in diameter) containing moistened cotton. After 24 h, 10 eggs per disc were sprayed with the EE (LC_{99}), Abamectin (100 ml/100L) and Tween 80 distilled water (control), in Potter tower.

Twenty replicates per treatment were used. The evaluation of hatching of larvae was checked daily until 144 h after installation of the experiment. The data were subjected to variance analysis and means compared by Tukey test, at 5% probability, using the 7.7 Assistat beta version (Silva and Azevedo, 2009).

Residual effect

Jack bean plants of 12 days-old grown in a greenhouse (29 ± 1°C and 60 ± 5 to R.H.) were sprayed with a volume of 26 ml/plant of either ethanolic extract (LC_{99}), Abamectin (100 ml/100 L) or Tween 80 distilled water (10 plants per treatment). After periods of 0, 24, 48, 72, 96 and 120 h after application (HAA), leaf discs (5.0 cm in diameter), were collected and in laboratory, exposed to 10 adult females of *T. urticae.*

The mortality was assessed daily until 120 h after confinement (Schlesener et al., 2013). The data were subjected to variance analysis and means compared by Tukey test at 5% probability, in a factorial arrangement (3 × 6), using Assistat 7.7 beta (Silva and Azevedo, 2014).

RESULTS

The EE of *A. muricata* seeds have shown the highest toxicity to *T. urticae*, as it required lower concentration (1.78 mg/ml) to cause 50% mortality, followed by EH and EA, with LC_{50} estimated in 3.29 mg/ml and 151.74 mg/ml, respectively. The values of LC_{99} were 933.38 mg/ml to EA, 31.87 mg/ml to EH and 12.07 mg/ml to EE (Table 1).

The Abamectin treatment caused 40% mortality in a commercial dosage of 100 ml/100 L. Since the EE proved to be the most efficient in mortality of *T. urticae*, it was selected to evaluate its effect as repellent, toxicity on eggs and its residual effect.

The lowest concentrations tested LC_{10}, LC_{20} and LC_{50}, and the Abamectin presented neutral effect on *T. urticae*, not being efficient in repellency spider mite (Table 2). By contrast, the concentrations equivalent to LC_{75}, LC_{90} and LC_{99}, respectively, presented repellent effect on *T. urticae* (Table 2).

The viability of the eggs of *T. urticae* was affected by the application of EE (LC_{99}). Only 9.5 ± 2.8% of the eggs were viable in contact with the LC_{99} dosage. Significant differences was observes among the used extracts (F = 139.2; P < 0.001). The Abamectin presented 76.5 ± 5.25% of viable eggs and the control 91.5 ± 2.43% (Figure 1).

Significant differences were observed on the mortality of *T. urticae* among the used treatments in the following h after application (HAA) (F = 3.57; P < 0.001), with the

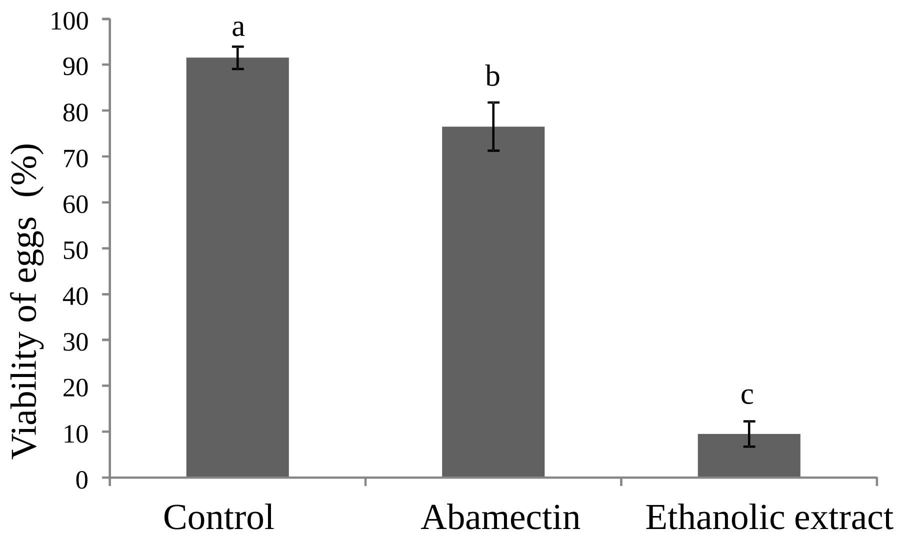

Figure 1. Viability of eggs of *T. urticae* exposed to LC_{99} of the ethanolic extract of *Annona muricata* seeds and Abamectin (100 ml/100 L).

exception of the period up to 72 HAA, showing no significant difference between the Abamectin and the control (Table 3).

In the period 0 HAA, it was observed that mortality of *T. urticae* was 93.3; 47.3 and 10.7% for EE, Abamectin and control, respectively. The EE had residual effect until the end of the experiment, 120 HAA with mortality above 80%. Abamectin afforded mortality rate of 12.7% 72 HAA (Table 3).

DISCUSSION

According to the obtained results, it was found that the ethanolic extract of *A. muricata* caused higher mortality for both LC (50 and 99), followed by hexanic extract mortality values. However, it is observed that the water does not work as efficient extractor for active ingredients of the plant in question.

The difference in the activity of aqueous and organic extracts (hexanic and ethanolic) is associated with the type of solvent (Trindade et al., 2000). Generally, the aqueous extract requires higher concentrations to cause pest mortality.

The acaricide effect of *A. muricata* extract is due to the presence of acetogenins, substances when used against arthropods, acted upon inhibiting the mitochondrial electron transport, affecting the action of NADH-ubiquinone oxidoreductase, which ultimately caused the death of these bodies (Álvarez et al., 2007).

Higher concentrations of EH were required to cause mortality of 50% of the *T. urticae* population compared to EE. These results seem to be related to the polarity of the solvents. Generally, non-polar solvents, such as hexanic (polarity 0.1) are less efficient than those with intermediate polarity, like ethanolic (polarity 4.3) (Potenza et al., 2005).

Other extracts and essential oils of botanical species were also efficient in controlling *T. urticae* (Ismail et al., 2011; Roh et al., 2011; Schlesener et al., 2013), these studies have shown that natural acaricides can be a good alternative to replace or reduce the use of chemical pesticides and, thus, mitigate the negative impacts that they cause.

Beyond the lethal toxic effect, other parameters have also been observed in this study showing the effects as repellent and ovicide. The results allow to understand that, even at concentrations below that able to cause 99% mortality in mite population, (LC_{99}); the extracts may cause repellence in the populations that may have survived its toxic effect. Essential oils of other botanical species, *Mentha longifolia* and *Salvia officialis* (Lamiaceae) and *Myrtus communis* (Myrtaceae) in addition to their lethal effect, they were also repellents to *T. urticae* with RC_{50} values estimated at 147.47; 138.80 and 164.41 µl/L, respectively (Motazedian et al., 2012).

The most significant benefit of using the ethanolic extract of *A. muricata* to control spider mite was the ovicide effect. In this case, it is observed that lethal effect was greater than 80%, this effect can be explained by the presence of acetogenins, substances with insecticidal activity acaricide, which are found in greater amounts in the seeds of *A. muricata* (Álvarez et al., 2007).

Natural herbal products based on azadirachtin and rotenone also caused egg infeasibility, evidencing that botanical acaricides are efficient in the control of eggs hatch of *T. urticae* (Brito et al., 2006; Duso et al., 2008). The ovicidal effect of an acaricide is a relevant property, because by controlling the initial stages of development of the mites, it may reduce or end the outbreak of

Table 3. Mortality of *T. urticae* is up to 120 h after application (HAA) ethanolic extract of *Annona muricata* seeds and Abamectin (100 ml/100 L).

Parameter	Mortality ± SE					
	0 HAA*	24 HAA	48 HAA	72 HAA	96 HAA	120 HAA
Control	10.7 ± 2.3^{cA}	9.0 ± 1.8^{cA}	10.7 ± 2.3^{cA}	6.0 ± 1.3^{bA}	8.0 ± 2.2^{cA}	4.7 ± 1.6^{cA}
Abamectin	47.3 ± 5.3^{bA}	32.0 ± 3.3^{bAB}	33.3 ± 4.9^{bAB}	12.7 ± 3.0^{bC}	23.3 ± 3.3^{bBC}	24.6 ± 5.8^{bBC}
Ethanolic extract	93.3 ± 3.3^{aA}	83.3 ± 6.9^{aA}	80.0 ± 4.8^{aA}	94.0 ± 4.1^{aA}	85.3 ± 6.1^{aA}	92.0 ± 4.3^{aA}
CV (%)	37.66					

Means followed by the same lower case letters in columns and capital letters on lines do not differ by Tukey test (P ≤ 0.05). *HAA: Hours after application; CV: coefficient of variation.

larvae and reduces the injuries caused by the pest (Esteves Filho et al., 2008).

The ethanolic extract of *A. muricata* seeds caused mortality during the 120 HAA, that is, throughout the experiment. Studies carried out with extracts of *Dieffenbachia brasiliensis* Veitch. (Areceae), *Ruta graveolens* L. (Rutaceae), *Allium cepa* L. (Liliaceae), *Agave angustifolia* Haw (Amaryllidaceae) and *A. squamosa* L. (Annonaceae) also reduced the population of *T. urticae* in 86.87, 83.95, 80.97, 76.30 and 75.40%, respectively, in plants of *Phaseolus vulgaris* L., within seven days after application (Potenza et al., 2006), similarly to the results obtained in this study.

The ethanolic extract of *A. muricata* proved to be a promising way to control *T. urticae*, showing satisfactory results in mite mortality, toxicity on eggs, repellency and residual effect.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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Use of atmometers to estimate reference evapotranspiration in Arkansas

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Evapotranspiration (ET) data from atmometers were compared against evapotranspiration estimated by the FAO-56 Penman-Monteith equation, recommended method, in order to evaluate the accuracy of atmometers. Measurements by 3 atmometers with grass cover and 3 atmometers with alfalfa cover were compared, for one growing season, to Penman Monteith based grass and alfalfa equation–($ET0_{\_PM}$ and $ETr_{\_PM,,}$ respectively). Comparison between cumulative Evapotranspiration measured by atmometers and $ET0_{\_PM}$ or $ETr_{\_PM}$ showed that Atmometers, for both grass and alfalfa, underestimate evapotranspiration by 12.5-21 and 15% respectively. The three Atmometers with alfalfa cover give the same cumulative value (636 mm) compared to the atmometers with grass cover which exhibit different results (atmometers 1 and 3 (467 mm) and atmometers 2 gives 419 mm). Correlation between ET from atmometers and $ETr_{\_PM}$ or $ET0_{\_PM}$ estimates were generally good. Evaporation from atmometers with alfalfa cover showed the highest correlation to $ETr_{\_PM}$ (R^2 varying from 0.68 to 0.72) whereas evaporation from atmometers with grass cover present the lowest correlation (R^2ranges from 0.49 to 0.68). The results indicated that with the proper regression equation and a good calibration, atmometers could be used to estimate ET for crop water requirement where evapotranspiration estimates are not available from weather stations.

Key words: Atmometer, evaporation, evapotranspiration, irrigation, Penman-Monteith equation.

INTRODUCTION

In Arkansas, groundwater withdrawal for irrigation doubled from 1980 to 2000 (Winthrop Rockefeller Foundation, 2008). The same report highlighted that 73% of Arkansas water withdraw were used for irrigation and 80% of the water used for irrigation was groundwater. As a result, irrigation is the main activity contributing to the

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increasing of water withdrawal (Valipour 2015a, b). Therefore, particular attention has to be taken in order to better manage irrigation and estimate accurately the crop water requirement.

Reliable estimation of crop water requirements is very important and vital where water resources are limited and crops are constantly under the influence of low rainfall and high temperature (Tabari et al., 2013). Therefore, accurate quantification of crop water requirements is needed for optimizing water productivity, efficient use of water resources and improving management practices to reduce surface and groundwater deterioration (Irmak et al., 2006; Al Wahaibi, 2011; Valipour 2014a, b; 2015c).

The evapotranspiration (ET) is generally used for estimation of crop water requirement. Thus, as mentioned by Jia et al. (2013), knowledge of ET is very important for water management and water resource planning. Different methods are developed for estimating ET. Most of them use equations to determine the value of ET at daily, weekly, monthly, or seasonal basis. These equations use weather variables as inputs such as solar radiation, air temperature, wind speed, and relative humidity (Irmak et al., 2005, Valipour 2014c, d).

Among these methods, The Penman-Monteith model is the most accurate and widely used. The Food Agriculture Organization (FAO, 2015) and American Society of Civil Engineers (ASCE) have recommended it for use in irrigation management. However, it demands a lot of weather variables (Irmak et al., 2003) which could not be available everywhere.

The rice research center of University of Arkansas is using Atmometers in some of its fields, to determine ET for irrigation management and scheduling. The same technology has been installed in some farmer fields in order to know when and how much to irrigate. The results from Atmometers are judged accurate and very close to ETP Penman Montheith from some studies conducted in different regions: Hess (1996) and Knox et al. (2011) in England, Irmak et al. (2005) in Nebraska (USA), and Magluilo et al. (2003) in Mediterranean area.

The aim of this study is to compare the Evapotranspiration Penman- Monteith with the evaporation from atmometers (ET_gage) and to evaluate the seasonal variability between same atmometers of commercial types.

MATERIALS AND METHODS

The study was conducted at the Rice Research and Extension Center at Stuttgart in Arkansas (34°28'7.31"N, 91°24'56.14"W) at 62.2 m above mean sea level. Data of four months (May, June, July, and August 2013) of one meteorological station and 6 Atmometers (ET_gages of two types of covers: grass and alfalfa) were used.

Atmometers (Figure 1) are water-filled devices, in which the actual evaporation of water is measured over time. A graduated glass sight on the water supply tank allows the user to easily measure the evaporation that occurred over a given period. Distilled water was used to fill the cylindrical reservoir of each atmometer made of white PVC, which reflects the radiant energy and is less subject to temperature raising of the water. The individual readings taken from each atmometer (ET_{gage}) at the daily basis was determined by the difference between water levels on consecutive days. If readings are not taken for the week end, we have assumed reading Sunday = Saturday = (reading Monday - reading Friday)/2.

For each type of cover (grass and alfalfa), data from the three atmometers were compared in order to check their consistency. Evapotranspiration from Penman Monteith (ETO_PM) was calculated using the Equation (1).

$$ET_O \text{ or } ET_r = \frac{0.408\,(R_n - G) + \gamma(C_n/(T+273))u_2(es - ea)}{\Delta + \gamma(1 + C_d u_2)} \quad (1)$$

Where ET0 (Penman Monteith grass reference evapotranspiration) or ETr (Penman Monteith alfalfa reference Evapotranspiration) is in mm/day; Rn = net radiation at the crop surface (MJm_2 day_1); G = soil heat flux density (MJm_2 day_1); T = air temperature at 2 m high (°C); u2 = wind speed at 2 m high (m s_1); es = saturation vapor pressure (kPa); ea = actual vapor pressure (kPa); es-ea = saturation vapor-pressure deficit (kPa). Cn is numerator constant for reference type and calculation time step, and Cd is denominator constant for reference type and calculation time step For grass reference and daily step, Cn = 900, Cd = 0.34 and alfalfa reference, Cn = 1600, Cd = 0.38.

The Computer program Cropwat 8 was used to calculate ETo_PM (Allen et al., 1998) at the daily basis. Cropwat 8 is developed by FAO for the calculation of crop water and irrigation requirements based on soil, climate and crop data. Also, the program can be used to develop irrigation schedules for different management conditions and to calculate the water supply for different crop patterns (FAO, 2015). The inputs of the application are maximum and minimum air temperature, humidity relative, average wind speed, and percentage of daytime. The comparisons between Penman Monteith grass or alfalfa reference evapotranspiration ($ET0_{\_PM}$ or $ETr_{\_PM}$) and evapotranspiration from atmometers with grass or alfalfa cover ($ET0_{\_At}$ or $ETr_{\_At}$) were tested by fitting linear regressions.

$ET0_{\_PM}$ or $ETr_{\_PM}$ was considered as the dependent variables. The Student's test (t test) was applied to evaluate the significance of the intercept and the slope of the regression. All tests were performed at alpha = 1%. Also a 95% Prediction interval was determined and the regression was bounded by a lower and upper limit values. To evaluate the degree of agreement between evapotranspiration from the atmometers and ETP Penman, coefficients of determination (R^2) were calculated.

RESULTS AND DISCUSSION

Average monthly climatic information

Table 1 gives the average monthly climatic information from May to August 2013. It shows that the average temperature is the same for June, July and August. The month of May with 21°C presents the lowest value. The relative humidity is greater than 80% for May, July, and August and achieves its lowest value at June with a value of 76%. August presents the lowest average wind speed (1.22 m/s), solar radiation (19.9 MJ/m^2/day), and average hour sun (Hour).

To Penman Monteith and Atmometers (Grass)

A comparison between cumulative values of $ET_{\_At}$ and

Figure 1. Atmometer.

ET0__{PM} during the four months (June to August 2013) is shown in Figure 2. Cumulative ET0__{PM} is always greater than the cumulative values of ET__{At}. The ETO__{PM} exhibits a cumulative value of 526.2 mm. Atmometers 1 and 3 are very consistent and present slightly the same values, 462.7 and 462.5 mm respectively. In contrary, the atmometer 2 shows the lowest values (419.1 mm). These results highlight that atmometers underestimate the value of evapotranspiration during the growing season in Arkansas by 12.5% for atmometers 1 and 3 and 21% for atmometers 2. This result confirms the finding of Gavilán and Castillo (2009) in Spain and Alam and Trooien (2001) under semiarid conditions. Irmak et al. (2005) pointed out that rainfall may play a significant role in this underestimation because the wetness of the canvas cover and the membrane as well as the accumulation of rainwater would cause a reduction in the vapor pressure gradient between the plate surface and the surrounding air on rainy days. These results are different from those of Knox et al. (2011) and Alam and Elliott (2003) which showed that atmometers overestimate the value of evapotranspiration. Another study by Magliulo et al. (2003) in South Italy found that a slight underestimation of pan ET0 by atmometer. The difference can be explained by the climatic differences in these zones (Valipour, 2015d) or by a reading error (Dukes et al., 2004) because different persons were involved in the data collection and this fact can cause inconstancy in data reporting. The different values from atmometers 1 and 3 on one hand, and 2 on the other hand reveal that it may be by manufactory variability. Gavilan and Castillo (2009) revealed that may be a difference value from atmometer of same cover due sometimes to manufactory variability. It will be interesting to use these three same atmometers for long terms to see how they will perform.

Depending on the geographical area, the model, formula; or method used to calculate evapotranspiration, results are different compared to FAO Penman Monteith method (Snyder et al., 2005). Valipour (2015d) showed that Temperature based formula and temperature and relative humidity based formula overestimated Penman Monteith Evapotranspiration in some provinces in Iran.

Farmers use to irrigate, at average, every three to five days; therefore the mean of the five-day sum values of evaporation were computed using the atmometers and the Penman Montheith. Also, Magliulo et al. (2003) pointed out that for practical purposes, a weekly schedule in ET0 monitoring via atmometers is to be advised to

Table 1. Average monthly climatic information.

Variable	Month			
	May	**June**	**July**	**August**
Average temperature (°C)	21	26.5	26.4	26.4
Daily relative humidity (%)	80	76	81	85
Average wind speed (m/s)	2.89	1.97	1.46	1.22
Average daily income solar radiation ($MJ/m^2/day$)	21.5	22.8	22	19.9
Average hour sun (Hour)	7.9	8.5	8.2	7.3

Table 2. Comparison between 5 days-sum ETo_PM and ETO_At.

Variable	Atmometer ($ET0_{\_Atm}$)			Penman- Montheith
	Atmometer 1	**Atmometer 2**	**Atmometer 3**	**($ET0_{-PM}$)**
Mean (mm)	15.56	14.19	15.60	18.56
Standard deviation (mm)	4.09	3.47	4.07	2.42
Standard error	0.87	0.74	0.87	0.42
Coefficient of variation (%)	26	24	26	13
T test	-2.96	-4.85	-2.94	
P value	0.006	<0.001	0.006	

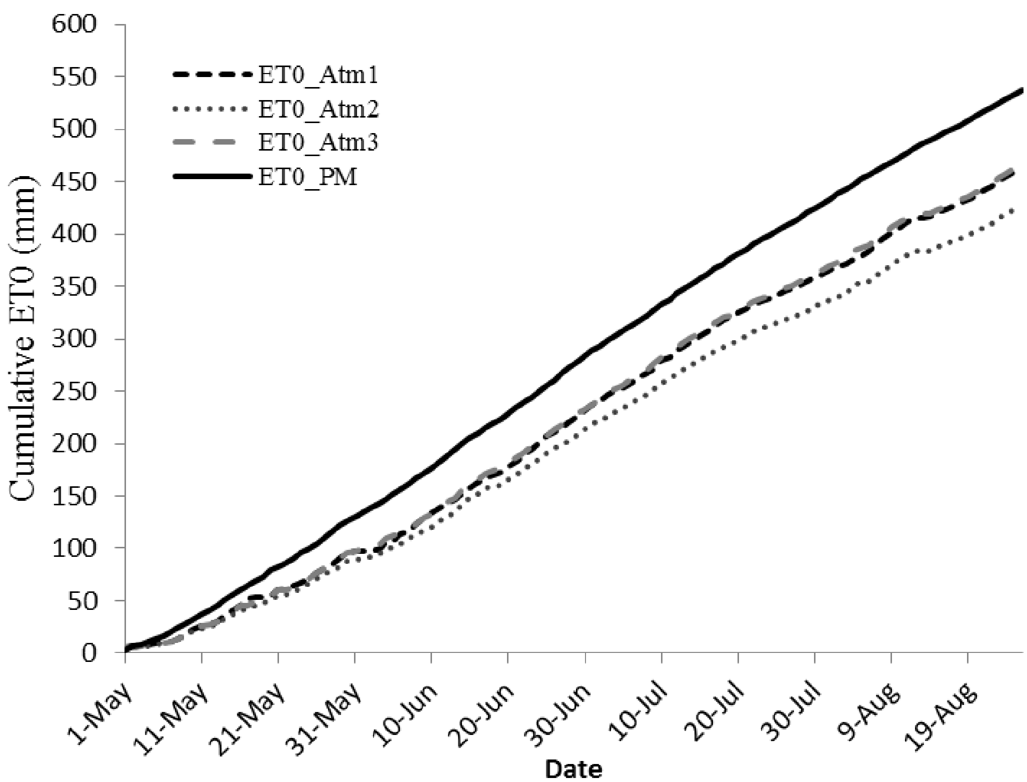

Figure 1. Cumulative potential evapotranspiration.

achieve best results, especially when manual instruments with visual reading are used. Here, we considered the week as the five days. The values calculated are shown in Table 2. In addition, Table 2 provides the standard deviation, the standard error, the coefficient of variation, and the value of t test. It can be seen that the mean

Table 3. Regression of atmometers reference grass and evapotranspiration Penman Monteith.

Atmometer	Regression slope	Regression intercept	R^2
Atmometer 1	0.41	12.1	0.49
Atmometer 2	0.57	10.4	0.68
Atmometer 3	0.49	10.9	0.67

ranges from 14.19 mm to 15.60 mm for the atmometers and 18.56 mm for the ETo_PM. The three atmometers yield higher standard deviation and error compared to the ETo_PM method. The t test shows that there is a significance difference between mean value from the atmometers and the Penman Montheith method (Pvalue <0.007). The ratio between average five days sum ET0_PM and ET0_At is 1.19, 1.31, and 1.13 for atmometers 1, 2 and 3 respectively.

The five-days sum evaporation values computed using the different methods (Penman Montheith and Atmometers) were analyzed by using a simple linear regression equation (Y = Ax +B) where Y represents ETo_PM and X values from the atmometers. A and B arerespectively the slope and the intercept of the regression. The results are shown in Table 3. There is good correlation ($R^2 > 0.65$) between atmometers 1, 3 and the ETO_PM but the correlation between ETO_PM and atmometer 2 shows a low R^2 value (0.49). This result confirms those shown above.

None of the regressions had a slope of 1 or an intercept of 0 (Table 3). All three slopes are less than 0.6 and statically different from 1 and the intercept is statistically different from 0 (Student's t-test at the 0.01 level). These results show that values from atmometers need to be calibrated before using them in irrigation scheduling. Most of the study comparing atmometers and the $ETo_{\_PM}$ showed that a calibration is needed Figure 3 presents the regression with a 95 % interval confidence. It shows that all the point fall in the confident interval showing an acceptable agreement between ET PM and ETO_At.

ETr Penman Montheith and Atmometers (Alfalfa)

Cumulative ETr_PM is greater than those of the three atmometers for all periods (Figure 4). The result reveals that the atmometers underestimate ETr. On the other hand the cumulative ETr of the three atmometers are nearly the same for the four Months (May to August 2013). This shows that the values from the three atmometers reference alfalfa are very consistent whereas the atmometers reference grass showed manufacture variability.

Table 4 gives the different statistics for the evapotranspiration from Atmometers alfalfa and Penman Monteith. The mean evapotranspiration reference is smaller for atmometers compared to Penman Monteith with high standard deviation. If we consider the atmometers; they have the same mean 21 m, 21.9 mm and 21.7 mm respectively and the same standard deviation and standard error.

The ratio between average five days sum ETr_PM and ET0_At is 1.19, 1.31, and 1.13 for atmometers 1, 2 and 3 respectively. The mean value of the ETr_PM five day average is significantly different from the mean of the 3 atmometers (Pvalue < 0.005). Like in grass atmometers, a five days sum Evapotranspiration has been calculated and regression on ET_PM against ETr_At is performed; the results show coefficient of determination more than 65% for all 3 regressions.

Figure 5 presents the different regressions on evapotranspiration from atmometers against Alfalfa reference evapotranspiration. Overall, all points fall in the area between the lower and upper band of a confidence interval of 95% except for one point which is not representative of the all data points. These results show that the atmometers based alfalfa give best estimation of the evapotranspiration compared to grass atmometers.

The atmometer 1 presents a lower $R^2 = 0.68$ compared to the atmometers 2 and 3 which show a R^2 of 0.71 and 0.72 respectively (Table 5). Overall, the three regressions present good correlation between ETr_At and $ETr_{\_PM}$ ($R^2 > 0.65$). The standard error estimates of the three regressions are relatively high with the highest value for atmometer1 (6.43 mm) which has also the lower R^2 (0.68).

Conclusion

This study evaluated the performance of 6 atmometers (3 with grass cover and 3 with alfalfa cover) to estimate reference evapotranspiration against the grass and alfalfa Penman Monteith Equation ($ETO_{\_PM}$ and $ETr_{\_PM}$, respectively) in Arkansas. Atmometers underestimated reference evapotranspiration during the growing season between 12.5 to 21%. Results obtained from comparison between 5-day ETgage measured by atmometers and estimated $ET0_{\_PM}$ or $ETR_{\_PM}$ using the FAO-56 Penman-Monteith equation showed a relative good correlation resulting in R^2 values varying between 0.48 and 0.72. Atmometer with alfalfa cover had better performance compared to grass cover. Manufacturing variability evaluation between atmometers of same cover showed that Atmometers with grass cover present some

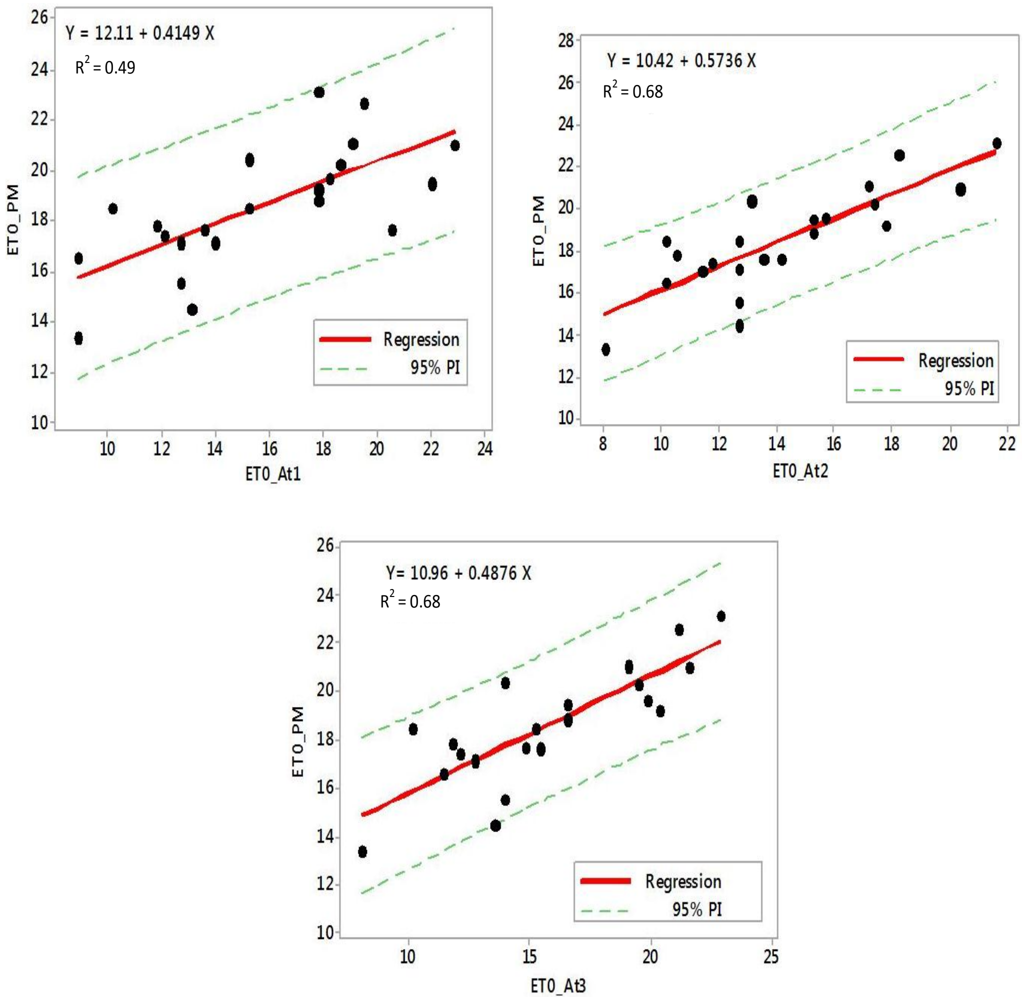

Figure 3. Correlation of Penman evapotranspiration to evapotranspiration measured by atmometers grass reference. The dashed lines show a 95% prediction interval.

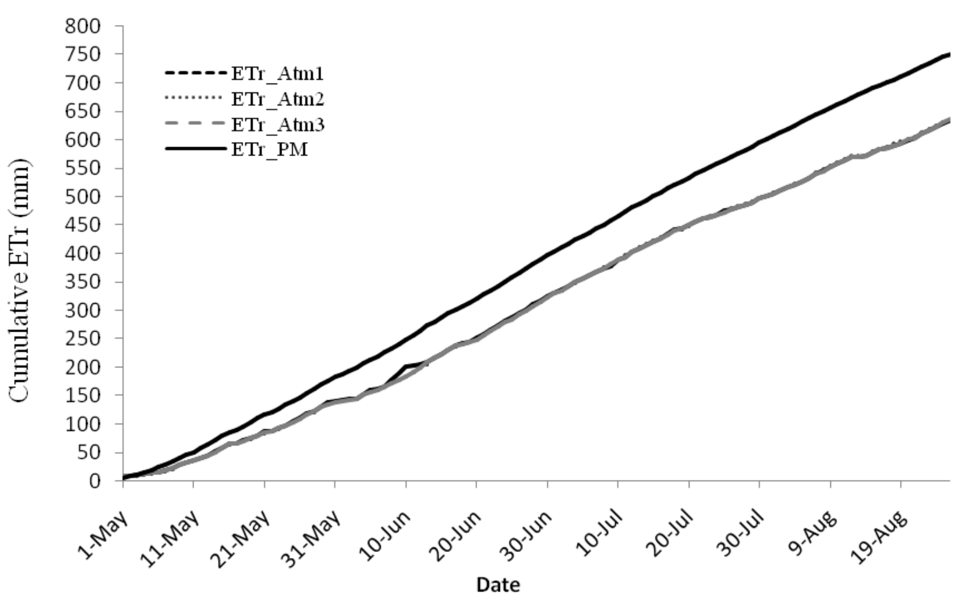

Figure 4. Cumulative potential evapotranspiration.

Table 4. Comparison between 5 days-sum ETr_PM and ETr_At.

Variable	Atmometer ($ETr_{\_Atm}$)			Penman- Montheith (ETr_{-PM})
	Atmometer 1	Atmometer 2	Atmometer3	
Mean (mm)	21	21.9	21.7	25.9
Standard deviation (mm)	5.5	5.3	5.5	3.39
Standard error	1.14	1.10	1.14	0.70
Coefficient of variation (%)	26	24	25	13
T	-3.67	-3.08	-3.10	
P value	0.001	0.004	0.004	

Table 5. Regression of atmometers reference alfalfa and evapotranspiration Penman Monteith.

Atmometer	Regression slope	Regression intercept	R^2	Standard error estimate (mm)
Atmometer 1	0.31	14.9	0.68	6.43
Atmometer2	0.54	14.2	0.71	4.96
Atmometer 3	0.52	14.71	0.72	5.2

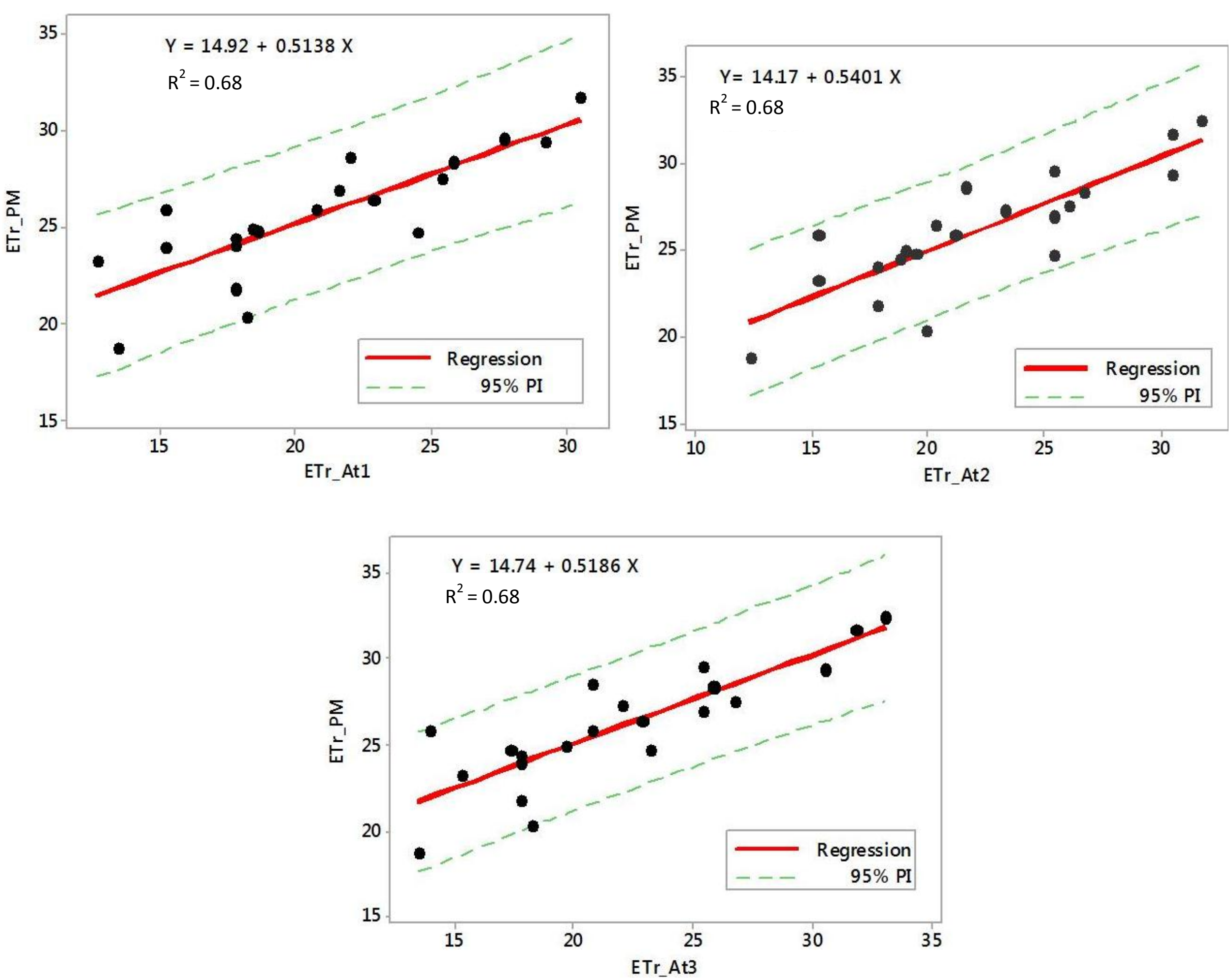

Figure 5. Correlation of Penman evapotranspiration to evapotranspiration measured by atmometers alfalafa reference Stuttgar, Arkansas. The dashed lines show a 95% prediction interval.

disparities. For grass cover, Atmometers 1 and 3 overestimated water losses as compared to atmometer 2. With a proper regression equation and a good calibration, atmometers could be used to estimate ET for crop water requirement where evapotranspiration estimates are not available from a weather station.

Conflict of Interests

The authors have not declared any conflict of interests.

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Full Length Research Paper

Agribusiness gross domestic product (GDP) in the Brazilian region of paraná and, the economic development of its agricultural cooperatives

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This paper presents the estimation and analysis of the agribusiness GDP in the Brazilian state of Paraná between 2006 and 2011, and its comparison with the economic development of the state's agricultural cooperatives in the same period. The GDP aggregates were detailed in the analysis that allowed assessment of the distribution of the respective inputs from agriculture, industry, and the distribution and services sectors. The results indicated the importance of each sector in the growth of Paraná's agribusiness and its share in the state's GDP. The impressive performance of Paraná's agriculture is evident as it grew 13.1% in 2007 and 32.4% in 2010. Historically, the distribution and services sector is the largest contributor to the agribusiness GDP, with about 13% representation. The industry sector was more sensitive to the events that followed the global economic crisis of 2008. Its share in the agribusiness GDP ranged from 10.3% in 2006 to 8.6% in 2011. In parallel, the revenue performance of cooperatives and the importance of the economic activity of the state through the use of financial resources and investments in industrialization are highlighted.

Key words: Agribusiness economics, Brazilian agribusiness, gross domestic product, Cooperatives of Brazilian State of Paraná.

INTRODUCTION

The concept of agribusiness (Gunderson et al., 2014) is comprehensive and includes, in addition to rural property, all other production, support, and agricultural distribution activities (CEPEA, 2014a; Ustriyana, 2015). It is a system of production chains that encompasses suppliers of materials and services, farms responsible for production

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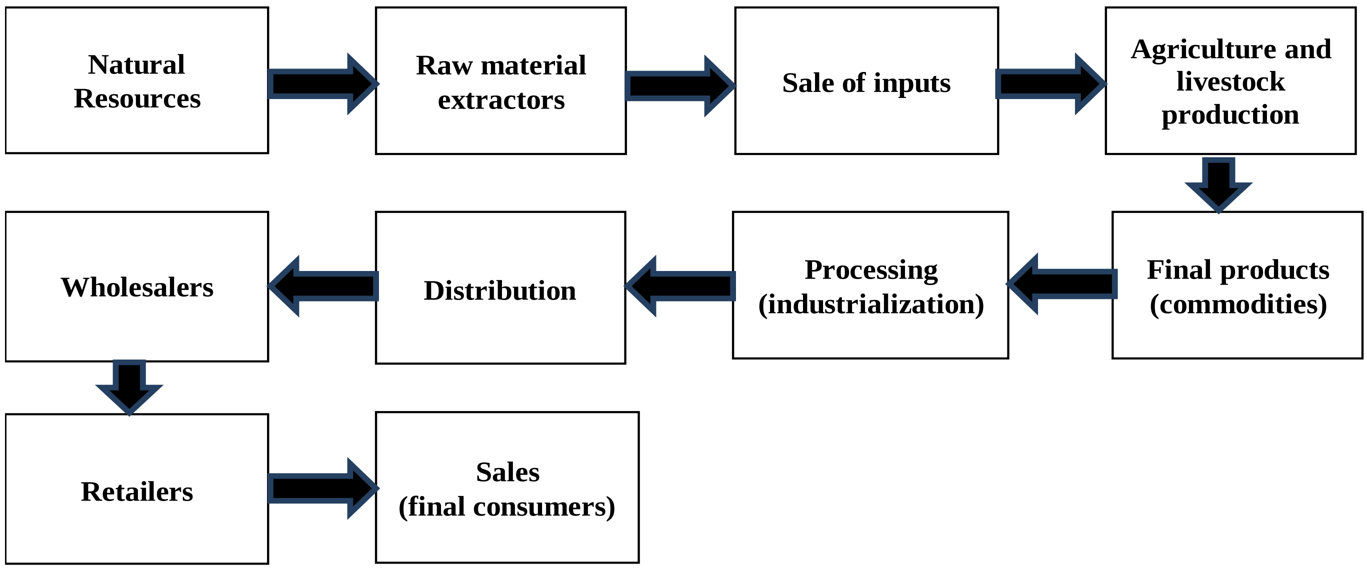

Figure 1. Agribusiness supply chain. Source: Author's.

(crops, livestock, plant extracts), storage, processing, and manufacturing industries, and distribution and marketing agents (Saragih, 2001; Veiga et al., 2014; Rocha et al., 2015). The interaction and influence among the links of the chain are critical in agribusiness conceptualization. The value addition of the industry's products goes through five distinct stages: (i) supply, (ii) production, (iii) processing, (iv) storage, and (v) distribution (Sawik, 2015; Lee et al., 2016).

According to the Brazilian Corporation of Agricultural Research (EmpresaBrasileira de PesquisaAgropecuária– EMBRAPA - https://www.embrapa.br), agribusiness is a network composed of several agents that are responsible for the production and sale of inputs, agricultural production, processing, distribution, and sale to the final consumer (EMBRAPA, 2005; Ustriyana, 2015) (Figure 1) illustrates agribusiness as a comprehensive supply chain.

The production and sale of inputs involve the extraction of raw materials, processing, and distribution, leading to sale for agricultural production. Agricultural production by small, medium, and large producers involves technical support, environmental management, and other direct and indirect aspects that are related to the generation of goods and services linked to the rural environment (Marine et al., 2016). Processing, distribution, and sale encompass industry, distributors, and consumers of agricultural products and services. Agribusiness also includes the institutional environment, which consists of the culture, traditions, education, customs, and the organizational environment composed of information, associations, research and development, and finance (EMBRAPA, 2005; Neves and Scare, 2010; Haggblade, 2011).

Agribusiness has always played a key role in the development of the Brazilian economy (Wilkinson et al., 2015). Brazil's economic upturns during the coffee, cattle, sugarcane, sugar, rubber, cocoa, and other "cycles" are proof of this industry's economic and social contributions (Gunderson et al., 2014). According to Guilhoto et al. (2000), the country's economic tradition in agribusiness is a trend that should prevail in the future, primarily because of the availability of its vast natural resources. The size of the Brazilian territory is 880 million hectares, where 388 million are arable, of which 90 million have not yet been explored (Portal, 2014). This availability of area, non-existent in most countries, coupled with the global growth in food demand, creates a positive scenario for the national agribusiness. The industry employs 38% of the country's workforce and accounts for about 40% of the volume of national exports (Martins et al., 2014). Agribusiness is an economic industry of vital importance to Brazil because it contributes a significant share of job creation, positively supports the trade balance with the strength and magnitude of its exports, and substantially influences the composition of the Brazilian GDP (Wilkinson et al., 2015).

Agricultural systems around the world are incrementally being dominated by vertically coordinated or integrated organizations (Gunderson et al., 2014; Purves et al., 2015). This is already a reality in developed countries and is being rapidly experienced in developing countries, especially in South America and Southeast Asia (Fao, 2005). Thus, farmers, especially the smallerones, should seek associations, cooperatives, alliances, or other forms of support to strengthen themselves and be able to play effectively significant roles (Guilhoto et al., 2000; Haggblade, 2011). At the rural level, membership organizations can be characterized by various types of structures. Among these are cooperatives, which are structured organizations that can achieve high levels of vertical integration.

This paper presents the agribusiness GDP of the state of Paraná (Southern Brazil region) in the years 2006 to 2011, and a comparison with the economic development of Paraná's agricultural cooperatives over the same period. The computation was made through an adaptation of the method used by the National Confederation of Agriculture (Confederação Nacional da Agricultura- CNA: http://www.canaldoprodutor.com.br) to calculate the agribusiness GDP in Brazil, which in turn is based on Furtuoso and Guilhoto's method (2003). The use of this method allows the comparison of Paraná's state results with CNA's. This method was also applied to the agribusiness GDP calculations of the states of Bahia, Espirito Santo, and Rio de Janeiro (Guilhoto et al., 2007; Bonelli et al., 2011; Barros et al., 2013). Although there are no specific studies to measure, in a disaggregated way, the role of the cooperatives' activities in the formation of Paraná's GDP, it is possible to demonstrate the economic development of cooperatives in strict relation to the evolution of Paraná's GDP.

MATERIALS AND METHODS

In order to calculate the GDP, it is necessary to consider the entire production chain. Thus, agribusiness is divided into four segments: Inputs, Agriculture and Livestock, Industry (agriculture-based), and distribution (transport, trade, and services). In each segment, the GDPs corresponding to the sectors of Agriculture (total production chains of crops and other plant activity) and Livestock (total production chains of animal products) are estimated separately and then aggregated according to the following classification: Aggregate I - Input; Aggregate II - Agriculture and Livestock; Aggregate III - Industry; Aggregate IV - Distribution. The end result of the agribusiness GDP is the sum of the four aggregates.

To calculate the GDP of Aggregate I, the values of inputs produced in agriculture and livestock were considered within the activity in order to avoid double counting. The mean consumption was measured using Paraná's 2006 input-output matrix, discounting input costs. For each input supplier sector, the added value coefficient at market price was calculated by the following expression:

$$AVC_i = AV_i / X_i \quad (1)$$

Where: AVC_i = added value coefficient at market price of sector i; AV_i = added value at market price of sector i; and X_i = production value of sector i.

The GDP of Aggregate I at market price is calculated by multiplying the input values (obtained from the input-output matrix) by the added value coefficient, according to the following expression:

$$GDP_I = \sum_{i=1}^{n} Z_i \cdot AVC_i \quad (2)$$

Where: GDP_I = GDP of Aggregate I (inputs) of agriculture, forestry, logging, and livestock and fisheries; Z_i = total value of input in sector i.

Aggregate II corresponds to the agriculture and livestock GDP, and the values are measured separately for agriculture and livestock. In the original method of Furtuoso and Guilhoto (2003), the added value of providing inputs to the agricultural sector alone is considered in Aggregate I. This procedure was not followed in the method used in this study. Thus, the added value of the input supply to only the agricultural sector remained in Aggregate II. The added value at basic prices plus net taxes for subsidies was considered to obtain the value at market price. This procedure was made possible due to the existence of these values in Paraná's 2006 input-output matrix. With this procedure, the value added at basic agricultural price corresponds exactly to the information published by the Brazilian Institute of Geography and Statistics (IBGE) in regional accounts. Below are the expressions used for these calculations:

$$GDP_{agric} = AV_{pmagric} \quad (3)$$

$$GDP_{liv} = AV_{pmliv} \quad (4)$$

$$GDP_{II} = GDP_{agric} + GDP_{liv} \quad (5)$$

Where: $AV_{pmagric}$ = added value of agriculture at market price; AV_{pmliv} = added value of livestock at market price; GDP_{agric} = agriculture GDP; GDP_{liv} = livestock GDP; GDP_{II} = total agriculture GDP.

To measure the GDP of agriculture and livestock-based industry, which constitutes the GDP of Aggregate III, the industrial segments that consume raw materials from agriculture, in Paraná's input-output matrix, were determined. The sectioned sectors were: Food and beverages, textiles, clothing items and accessories, leather goods and footwear, wood products –excluding furniture, cellulose and paper products, alcohol, pesticides, furniture, and products of various industries. To avoid double counting, the value of the supply of inputs to agriculture, computed in Aggregate I, was subtracted from the agricultural industry's added value at market price. Thus, Aggregate III may be calculated by the following expression:

$$GDP_{III} = \sum_{i=1}^{n} [AV_{pmi} - (Z_i - AVC_{pmi})] \quad (6)$$

Where: GDP_{III} = GDP of Aggregate III (agri-industry); AV_{pmi} = added value at market price of the agri-industry of sector i; Z_i = total value of the input from sector i; AVC_{pmi} = added value coefficient at market price of sector i.

The GDP of Aggregate IV is the share of agricultural GDP related to distribution and service. To that end, first the added value of trade, transport, and services were obtained. For services, the added value of the following service activities was considered: Information, financial intermediation and insurance, real estate and rent, boarding and lodging, and services provided to businesses. The added value at market price was calculated by adding the added value at basic price to net indirect taxes of subsidies on products. It was also necessary to consider the values of the final demand of the agribusiness segment when totaling the final domestic demand. Therefore, the final domestic demand was obtained using the following expression:

$$DFD = GFD – NITFD – IPFD \quad (7)$$

Where: DFD = domestic final demand; GFD = global final demand; NITFD = net indirect taxes paid on the final demand; IPFD = imported products on the final demand.

Thus, the GDP of Aggregate IV was calculated using the following expression:

Table 1. Paraná's agribusiness GDP, Paraná's total GDP, Brazil's agribusiness GDP, and Brazil's total GDP – in million R$.

Variable	2006	2007	2008	2009	2010	2011
Aggregate I –Inputs	1,742	2,127	2,830	2,548	2,251	2,345
Aggregate II –Agriculture	10,467	12,866	15,443	13,600	17,148	19,279
Aggregate II –Industry	14,087	14,466	15,370	14,416	17,361	20,819
Aggregate IV –Distribution and services	16,999	21,041	22,946	24,509	27,484	29,647
Total –PR Agribusiness GDP (+ PR GDP % share)	43,296(31.7%)	50,500(31.3%)	56,589(31.6%)	55,073(29%)	64,245(29.6%)	72,090(29.8%)
2011 PR GDP (IPARDES)	136,615	161,582	179,263	189,992	217,290	241,809
2011 BR Agribusiness GDP (CEPEA) (+BR GDP % share)	772,684 (23%)	833,666 (23.3%)	886,084 (23.5%)	834,316 (22.2%)	879,116 (21.8%)	917,654 (22.1%)
2011 BR GDP (IPEA)	3,372,239	3,577,656	3,762,678	3,750,271	4,032,805	4,143,013

Source: CEPEA (2014b), IPARDES (2014), IPEA (2014).

$$GDP_{IV} = (AVC_{pm} + TAV_{pm} + SAV_{pm}) \cdot \sum \frac{FD_i}{DFD} \quad (8)$$

Where: = AVC_{pm}= trade added value at market price; TAV_{pm} = transportation added value at market price; SAV_{pm} = services added value at market price; FDi = final demand of agribusiness activities; DFD = domestic final demand.

The total agribusiness GDP corresponds to the sum of the GDPs of the four aggregates. That is, the sum of the results of expressions (2), (5), (6), and (8):

$$GDP_{agribusiness} = GDP_I + GDP_{II} + GDP_{III} + GDP_{IV} \quad (9)$$

RESULTS AND DISCUSSION

Agribusiness corresponds to the set of activities related to agriculture, including input supply, processing, marketing, and distribution. Paraná's state agribusiness, thus defined, represented 31.7% of the state's GDP in 2006, varying to 29.8% in 2011. The importance of this activity is clear to the state's economy through its share in Paraná's GDP.

In current values, the GDP of Paraná's agribusiness was R$ 72.1 billion in 2011. Out of this, R$ 2.3 billion was the share of the input supply (Aggregate I). Agriculture and livestock (Aggregate II) contributed R$ 19.2 billion. For industrial activities (Aggregate III), the value of the gross domestic product was R$ 20.8 billion. Finally, distribution and services accounted for R$ 29.6 billion of the state's agribusiness GDP (Table 1) shows, for the period of 2006 to 2011, the values of Paraná's agribusiness GDP aggregates, the state's total agribusiness GDP and its percentage share of Paraná's GDP, Paraná's total GDP, Brazil's agribusiness GDP with its percentage share in relation to Brazil's GDP, and Brazil's total GDP.

Figure 2 shows the evolution of Paraná's agribusiness GDP and Brazil's agribusiness GDP in the period of 2006-2011, in million R$. An increasing trend over the period can be observed, with the exception of 2009, which showed a sharp decrease in the GDP of state and national agribusiness.

Figure 3 shows the percentage share of the agribusiness GDP in Paraná's total GDP. In the period 2006-2008, the agribusiness share in the state's GDP was above 31.0%, led by an exceptional increase in agriculture. The good performance of agribusiness presented in this period was, among others, a result of the cumulative growth of 26.03% in soybean production, 148.17% in wheat, and 38.91% in corn production (IBGE, 2006, 2008). In 2009, the state's economy declined by 1.32%. This negative performance was mainly due to the global economic crisis of 2008, which started in the US housing sector. The weakening of Paraná's economy had also generated negative effects on the performance of agribusiness. The highest decrease of agribusiness' share in Paraná's GDP was recorded in 2009, when the share dropped from 31.6% in 2008 to 29% in 2009.

Figure 4 shows the share of aggregates in the composition of Paraná's agribusiness GDP. It is clear that in 2009 the industrial activity and agriculture and livestock production were the most affected by the global economic crisis of 2008, showing decrease compared to the previous year. This scenario was caused mainly because the crisis impacted the domestic demand for agribusiness products and the exports of agribusiness products contracted. According to Suzuki Junior (2009), during January to September, 2009, exports of fresh chicken meat, crude soybean oil, and plywood showed sharp revenue declines of -23.5, -52.6 and -52.0%,

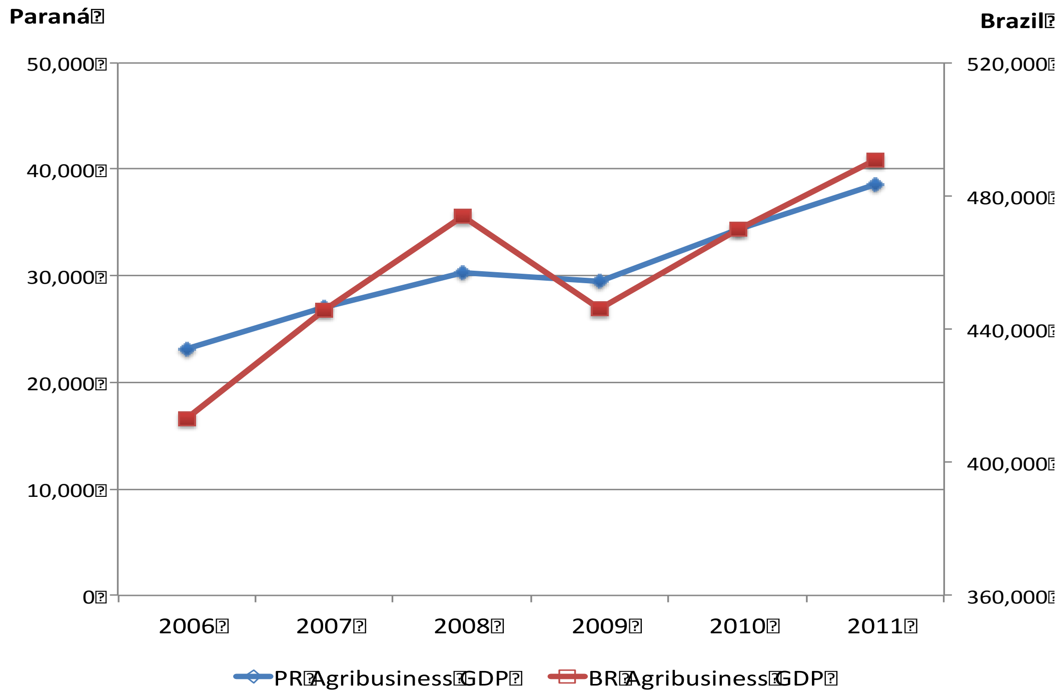

Figure 2. Agribusiness GDP evolution in Paraná and Brazil (R$ million 2011). Source: Author's.

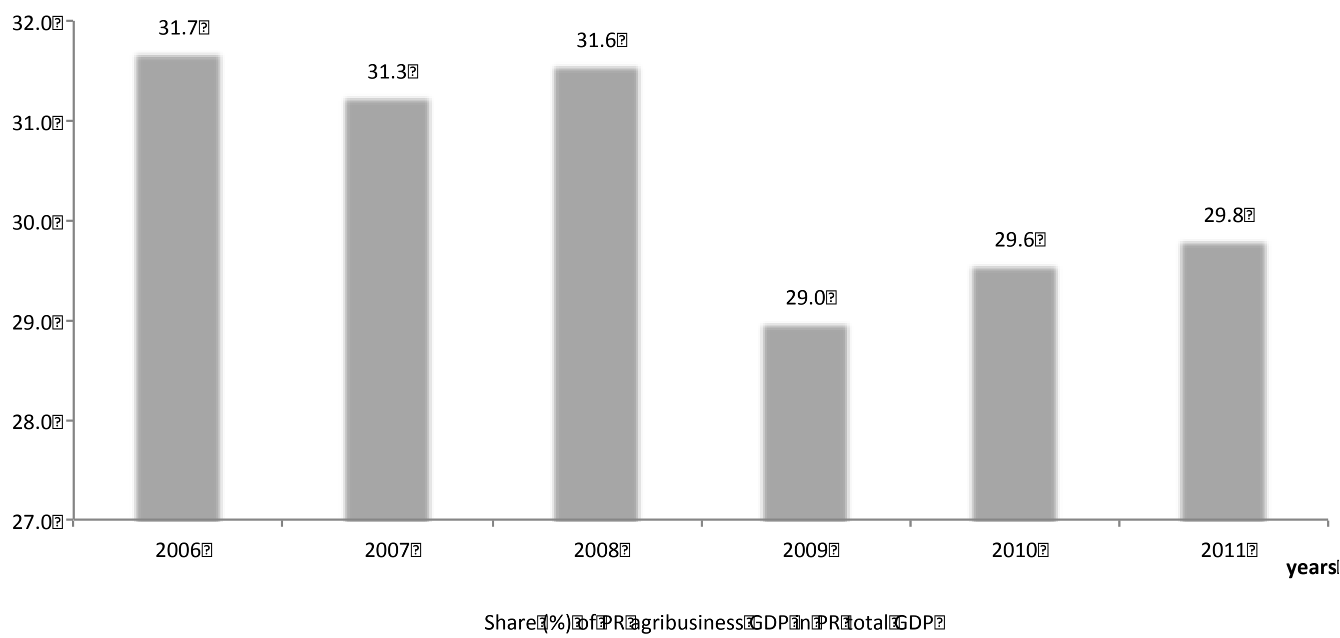

Figure 3. Percentage (%) share of the agribusiness GDP in Paraná's total GDP. Source: Author's.

respectively. However, it must be noted that agribusiness is recovering its share in Paraná's total economy, closing the year 2011 with 29.8% share, as showed in Figure 4.

Figure 5 shows the annual percentage variation of the agribusiness GDP and Paraná's total GDP from 2006 to 2011. It can be verified that the expansion of the real growth of the state's agribusiness GDP was higher than the state's total GDP growth in 2007 and 2010 and similar to the expansion of Paraná's economy in 2008 and 2011. This performance resulted in the expansion of

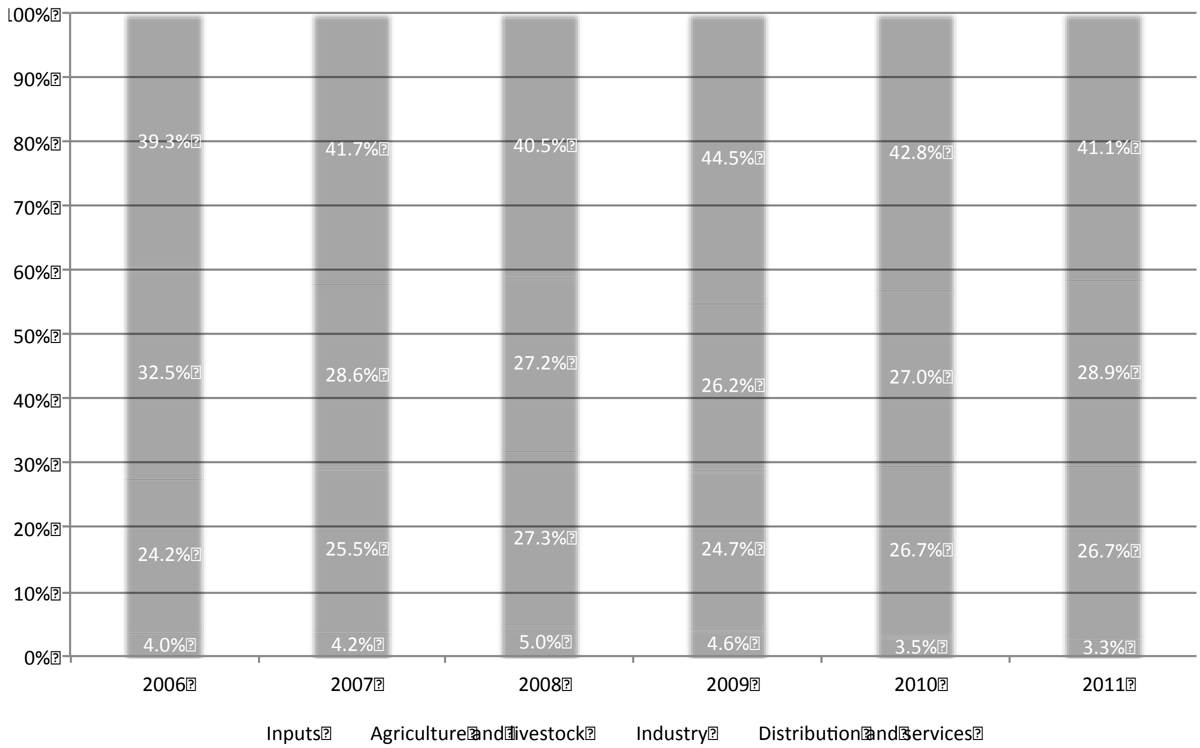

Figure 4. Share of aggregates in Paraná's agribusiness GDP. Source: Author's.

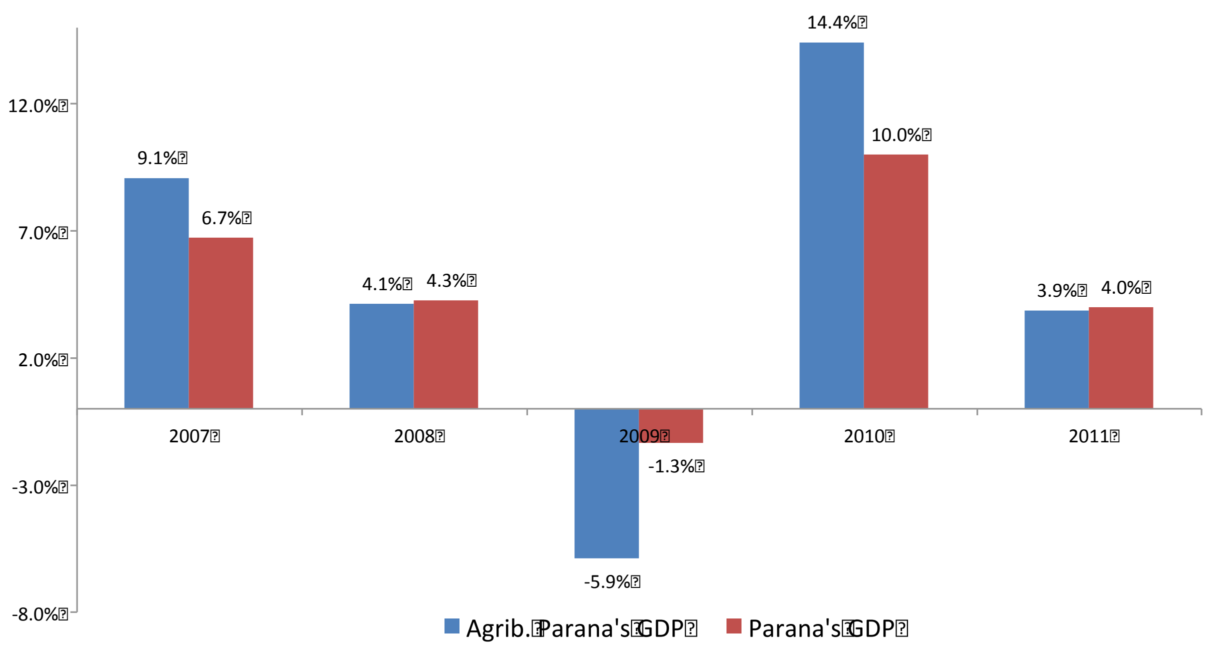

Figure 5. Annual variation of agribusiness GDP and Paraná's GDP. Source: Author's.

the labor market and of the local markets of the cities located on the Paraná agro-industrial regions. However, in 2009, the global economic crisis brought down the growth rate to -5.9%. This was mainly caused by the reduction in the volume of exports.

When assessing the annual variation of agribusiness

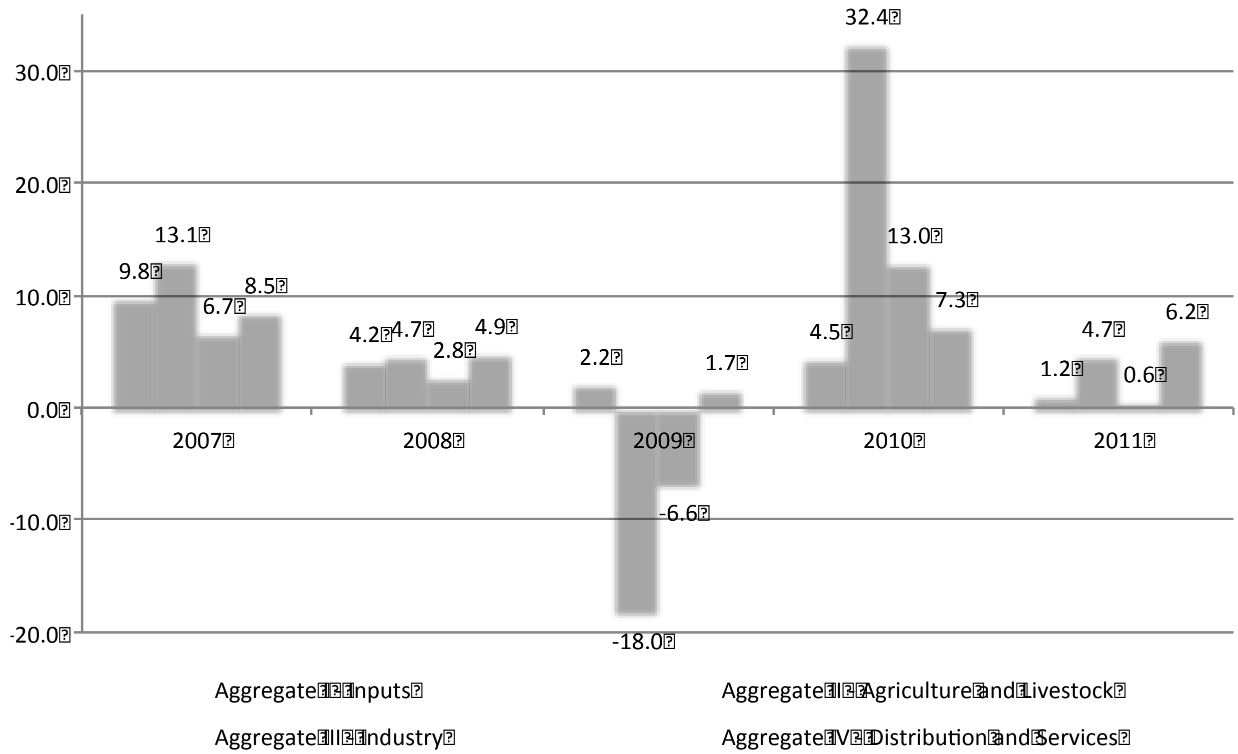

Figure 6. Annual % variation of Paraná's agribusiness GDP aggregates. Source: Author's.

GDP aggregates, shown in (Figure 6), it is possible to see the impressive performance of Paraná's agriculture and livestock (Aggregate II), expanding 13.1% in 2007 and 32.4% in 2010. In contrast, the 2009 results presented a variation of -18.0%. According to Suzuki Junior (2010), the negative performance of agribusiness in 2009 was mainly caused by climatic factors that led to crop failures. The severe drought that year resulted in a reduction of 22.8% in grain production. Another striking factor for agribusiness in 2009 was the performance of the timber segment, which showed production decline to the order of 22.7%. On the other hand, the inputs as well as the distribution and services sectors had positive variations throughout the analysis period, and they made a definite contribution to the growth of the state's economy.

Cooperatives in Paraná, Brazil

Paraná hasa significant representation of agricultural cooperatives. According to data from the Organization of the Paraná State Cooperatives (Organização das Cooperativas do Estado do Paraná –OCEPAR), agricultural cooperatives have a significant place in Paraná's agricultural economy, as they actively participate in the production, processing, storage, and industrialization processes of most agricultural products produced in the state (OCEPAR, 2010). According to Martins et al. (2014) agricultural cooperatives in Paraná serve as a model for the rest of the country. The authors note that, "In the context of Paraná, the cooperative stands as an instrument for the social ascension of members of the cooperative and also for the regional promotion and development based on highly competitive agribusiness chains."

Although there have been no specific studies to measure the role of the activities of cooperatives in the formation of Paraná's GDP in a disaggregated way, it is possible to demonstrate the economic development of cooperatives in strict relation with the evolution of Paraná's GDP. Figure 7 shows the growth of the total revenues of the state's agricultural cooperatives between 2006 and 2011, reaching R$ 26.5 billion by 2011. It is also possible to check the growth curve of Paraná's agribusiness GDP. The comparative analysis shows a correlation between the two periods. In this analysis, it is important to emphasize that the intention is merely to show the evolution of the indicators and the growth curve over time.

In Figure 8, the annual variation of these two indicators points to a higher positive variation for the total revenues of cooperatives in the first two, as well as in the last year.

The cooperatives' revenues are divided into sales of processed products, fresh grains, livestock products, sale of inputs for production, and sale of services.

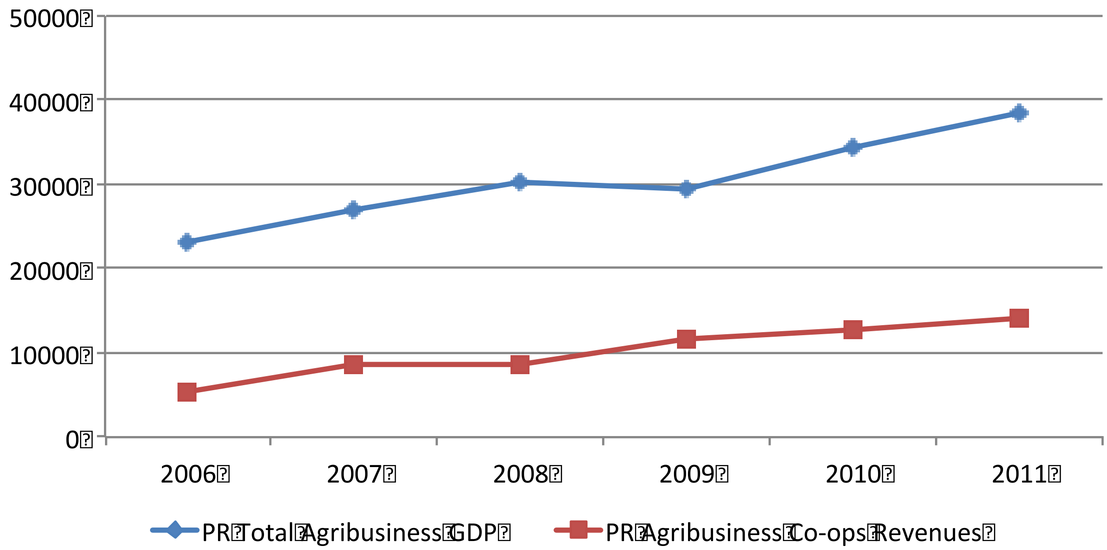

Figure 7. Evolution of Paraná's agribusiness GDP and total revenues of the state's agricultural cooperatives (R$ million/2011). Source: Author's own elaboration.

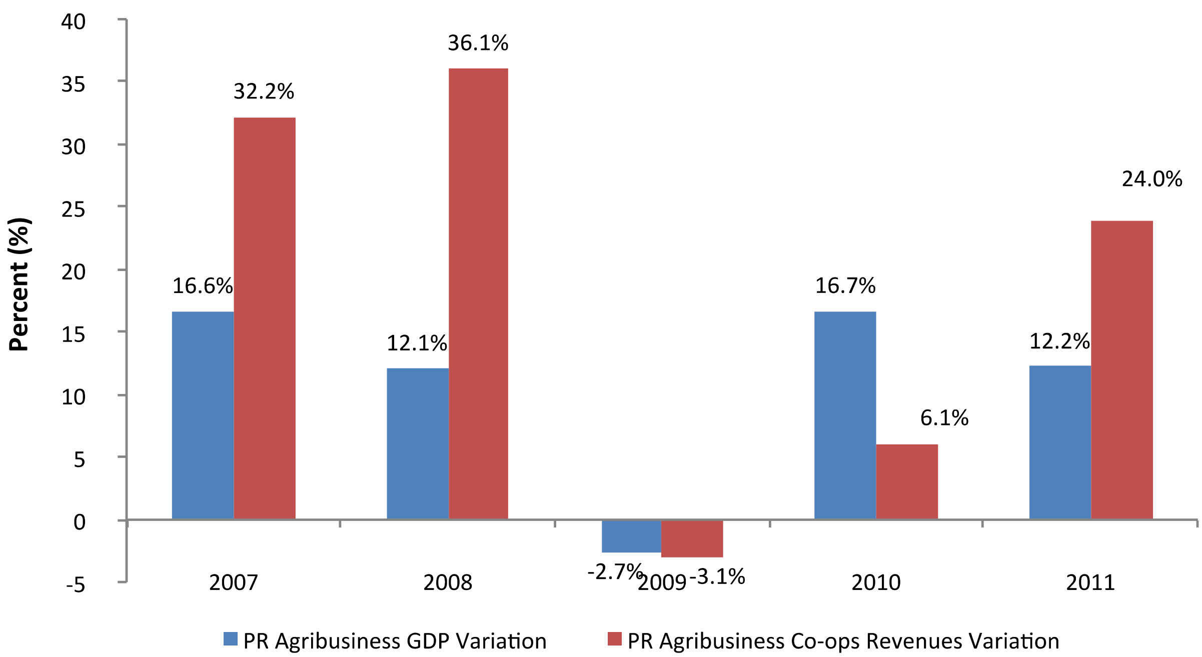

Figure 8. Annual variation of Paraná's agribusiness GDP and total revenues of Paraná's agricultural cooperatives. Source: Author's and OCEPAR (2014).

Figure 9 shows the stratification of income and the participation of these segments in total cooperative sales. The most representative segment is processed products (39%), reflecting the result of the high investments made by cooperatives in recent years with an aim to add value to their members' production. Some examples of products of this activity are: Oil refining, cuts of chicken meat, pork, UHT milk, powdered milk, sugar and alcohol, barley malt, feed, and various other retail items. Then, there is the sale of grain and livestock (37%), but that does not go through processing such as transfer of fresh grains for export and allocation of milk to non-cooperative industries. In addition, also highly represented is the sale of inputs (20%), mainly consisting of the sale of fertilizers,

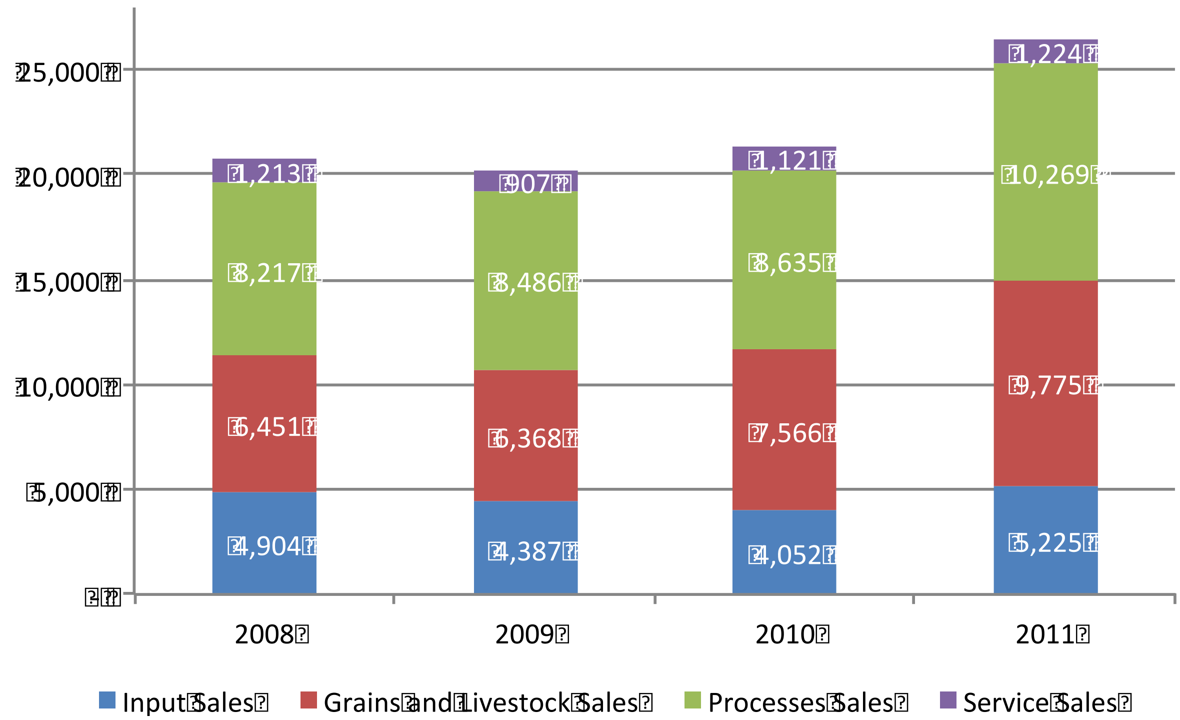

Figure 9. Revenues of Paraná's agricultural cooperatives by segment. Source: OCEPAR (2014).

correctors, and seeds made by cooperatives for the members' production. Finally, there is the sale of services (4%), which encompasses sales in supermarkets, gas stations, taxes, and other services.

As the largest portion of the sales of Paraná's cooperatives comes from industrial products, many investments have been made in this segment in recent years. The state has the highest volume of rural credit financing taken by cooperatives, for funding, investment, and sale of the production. Investments in infrastructure and agribusiness help sustain the growth of the sector, since production has been increasing continuously. Figure 10 shows the loans granted to the cooperatives in Paraná as compared with the total loans granted to cooperatives in the rest of the country. In 2011, Paraná received 28.8% of total funding specifically granted to cooperatives across the country, representing about 6% of Paraná's agribusiness GDP.

Conclusion

This article tries to present the estimation and analysis of the agribusiness GDP in the Brazilian state of Paraná between 2006 and 2011, and its comparison with the economic development of the state's agricultural cooperatives in the same period. The results indicated the importance of each sector in the growth of Paraná's agribusiness and its share in the state's GDP was observed. Agribusiness is an activity that has, historically, a large share in the national economy. In the period 2006-2011, the representation of agribusiness hovered around 23%. This representation is even higher in the economy of the state of Paraná. In the period evaluated in this study, agribusiness accounted for about 30% of the state's GDP. This study presented the calculation of the agribusiness GDP of the state of Paraná over the period 2006 to 2011, and a comparison with the economic development of the state's cooperatives. The calculation was made through an adaptation of the method used by the CNA for calculating the agribusiness GDP in Brazil.

The use of this method allowed comparison of Paraná's results with those of the CNA. This methodology was also applied to calculations of the agribusiness GDP of the states of Bahia, Espírito Santo, and Rio de Janeiro. In Paraná, the 2006 input-output matrix, provided by the Paraná Institute for Economic and Social Development (Instituto Paranaense de Desenvolvimento Econômico e Social (IPARDES), was used. By analyzing Paraná's agribusiness GDP aggregates in relation to the inputs, agriculture and livestock, industry, and distribution and services sectors, it was possible to verify the share of each sector in the GDP composition. Historically, the distribution and services sector has been the largest contributor to the GDP, with a share of about 13%. The input and agriculture and livestock sectors' shares remained with a low variation as compared with the agribusiness GDP over the period. The industry sector was more sensitive to the events that led to the global

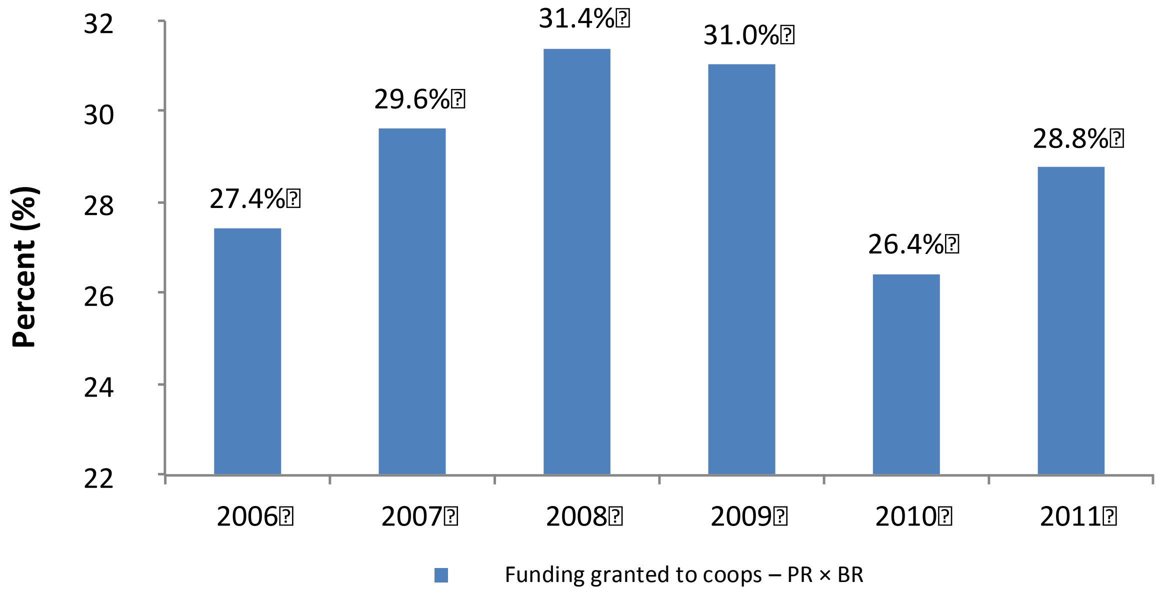

Figure 10. Funding granted to cooperatives of PR x BR. Source: OCEPAR (2014).

economic crisis of 2008. Its share in the agribusiness GDP ranged from 10.3% in 2006 to 8.6% in 2011. However, the variation in the share has shown to be positive. Comparing the agribusiness GDP variation with the state's total GDP variation, it was found that the sector had a relatively higher increase than the growth of the state's economy in 2007 and 2010. In the years 2008 and 2011, the growth of agribusiness followed the growth of the state's economy. In 2009, at the height of the global economic crisis, the impact on agribusiness was higher than the impact on the state's economy as a whole. The decrease in the agribusiness GDP was caused mainly by the decrease in the volume of exports and climate issues that led to significant crop loss.

Conflict of Interests

The authors have not declared any conflict of interests.

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