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Full Length Research Paper

Leaf content of macronutrients in *Tabebuia aurea* seedlings grown on different substrates and luminosities

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Taking into consideration that species react differently to the substrate and luminance variations, this study aimed to evaluate the leaf content of some macronutrients and dry matter in *Tabebuia aurea* seedlings through the hypothesis that the growth environment can alter the concentration of chemical elements in the species. The study was conducted in a greenhouse, in randomized blocks, factorial 2 × 3 (two light intensities and three types of substrates). The first greenhouse was covered with shade cloth, with maximum flow of 950 µmol m^{-2} s^{-1} radiation, and the second was 750 µmol m^{-2} s^{-1}. The substrates were: 1) Cerr. Savannah soil, 2) Ca-Mg – Savannah soil with calcium carbonate and magnesium and, 3) OM – Savannah soil with organic matter (2/1), with samples taken after 230 days of experiment. The data obtained indicated that *T. aurea* species has better development when grown in brighter locations in OM soils, which would be expected due to its higher nutritional content. However, when the seedlings were placed in soils with low nutrient content (Ca-Mg and Cerr.) and similar levels of potassium, plants maintained in soils with higher amounts of calcium and magnesium obtained higher growth, with greater accumulation of dry matter in leaves and total. This could indicate that the difference in growth should be related to increased amount of these Ca-Mg elements of treatment and the calcicole characteristic of the species tested.

Key words: Greenhouse, seedling formation, dry matter, ipê.

INTRODUCTION

The species *Tabebuia aurea* (Silva Manso) Benth. & Hook.f ex. S. Moore is found in areas of the Amazon region, Northeast, Midwest and Southeast, in different environments. The species is popularly known as “ipê”, “paratudo”, “caraíba”, “ipê-do-cerrado”, and “ipê-amarelo”, among other regional names and often found in the Pantanal (floodplain) and Cerrado (savannah) areas in the state of Mato Grosso do Sul (Lorenzi and Matos, 2008; Soares and Oliveira, 2009). Its wood is used for making tools, furniture and civil construction, among other uses and may be employed in landscaping; also it has use in popular medicine, to combat influenza, diabetes, malaria and inflammation in general, amongst other activities (Lorenzi and Matos, 2008). It is a species that has great potential for use, including Agroforestry Systems (AFS), in grazing associations to forest and non-forest species, in order to increase the production per unit area, with higher productive diversity in ownership (Rodrigues et al., 2007). Currently, there is a search for native tree species that can be used in these systems, with a significant number of plants of Cerrado and Pantanal region which may be used for this purpose.

However, for its use, the production of seedlings is necessary, using different environmental conditions such as luminosities and substrates. Regarding substrates, the correct choice of the appropriate material is important for producing high quality seedlings, as it directly affects the quality of plants due to the variation of physical, chemical and biological properties, and should have good porosity, water retention capacity, cationic change and uniform composition.

Furthermore, the diversity of plant responses regarding its growth associated with light is a factor which can affect the initial growth. According to Larcher (2003) and Lambers et al. (2008) the study of the behavior of the species in different light regimes assists in the study of its feasibility for use in reforestation or cultivation, among other actions. Light and its intensity and wavelength, among other characteristics, has great effect on plant growth, and the modification of these parameters may change plant growth (Larcher, 2003; Lambers et al., 2008; Taiz et al., 2014).

Due to its great potential for use, there are studies relating to some of the growth characteristics of the species *Tabebuia aurea* in different types of substrates and luminosities (Oliveira and Gualtieri, 2011, 2012; Oliveira and Perez, 2012), but none is related to accumulation of nutrients in their leaves with different environmental factors. According to Mengel and Kirkby (2001) and Marschner (2012), the concentration of foliar nutrients may vary, among other factors, in accordance with the light available and the type of soil used for seedling growth. It is also necessary that the foliar nutrient content be appropriate, allowing the seedlings a better rate of survival and adaptation to the environment, and allows the chemical profiling of leaves, important for the diagnosis of the nutritional status of plants.

Given the importance of knowledge about the processes of growth of native species, the objective was to evaluate the leaf content of some macronutrients and dry matter of seedlings grown on different substrates and luminosities, identify the factor that most interfered in development and correlate the data obtained with the calcicole characteristics of the species.

MATERIALS AND METHODS

Fruit collection

Tabebuia aurea fruits were harvested from 10 matrices located in Passo do Lontra, sub-basin of the river Miranda (Upper Paraguay River Basin), Municipality of Corumbá, Mato Grosso do Sul, and transported to the city of São Carlos, State São Paulo. After opening the fruits by natural dehiscence, the seeds were chosen with no apparent signs of attack by insects and equivalent size. Later, they were placed to germinate in plastic boxes containing filter paper moistened with Captan fungicide solution (0.2%) and kept on laboratory shelves under environmental temperatures in Plant Physiology laboratory of the Federal University of São Carlos (UFSCar). After issuing the primary root, the seedlings were placed in aluminium trays containing vermiculite, irrigated daily with distilled water and, after reaching a height of 7 cm above the substrate, transplanted to planting bags (black bags of 28 × 40 cm - width × height - with of 8 L capacity containing substrate, each recipient received single plantlet), and transported to greenhouses.

Study area and climate

The region where the experiment was developed has a Cwb type climate (dry-winter highland climate), according to Köppen classification, with well-defined seasons, with a dry, from April to September, and a rainy season, from December to February (tropical region). The climate is characterized as tropical, due to the seasonal rhythm of rainfall, under hot (with an average annual temperature below 22°C and at least one month with an average temperature below 18°C) and humid (short dry season in winter), with annual rainfall averages between 1,200 and 1,500 mm. Taking into account a sunny day without clouds, the first batch of plants had been kept in the house of vegetation covered with shade cloth (shading), with maximum passage of 950 µmol m^{-2} s^{-1} radiation (79.2%) and the second, in a greenhouse with maximum flow of 750 µmol m^{-2} s^{-1} luminosity (62.5%). The brightness values were obtained using a portable infrared gas analyzer (ADC-LCA-2).

Substrate

The substrate utilized, typic hapludox, sandy texture (68.3% sand, 5.1% silt and 26.6% clay), was collected on campus UFSCar, in Cerrado area (savannah), at a depth of 0 to 20 cm, sieved and placed on plastic canvas to dry outdoor. Based on collected soil, three types of substrate were prepared: 1) OM - Savannah soil with added organic fertilizer (manure) in the ratio 2: 1; 2) Ca-Mg -

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Table 1. Soil and chemical composition (Cerr. savannah soil; Ca-Mg - savannah with calcium carbonate and magnesium; OM – savannah with organic matter).

Substrate	OM ($g\ kg^{-1}$)	pH	P resin ($mg\ dm^{-3}$)	K^+	Ca^{2+}	Mg^{2+}	H + Al	Al^{3+}	SB	V%
				$cmol_c\ dm^{-3}$						
Cerr.	20.7	4.33	02	0.09	0.72	0.56	3.4	0.35	1.37	29
Ca-Mg	27.1	5.91	03	0.10	2.68	2.32	2.2	0.03	5.1	70
OM	36.5	4.89	22	0.58	1.24	1.08	2.6	0.14	2.9	53

(OM - organic matter, pH in $CaCl_2$, P - phosphorus, K^+ - potassium, Ca^{2+} - calcium, Mg^{2+} - magnesium, H + Al – potential acidity, Al^{3+} - aluminium, SB – sum of bases, V - saturation of bases).

Savannah soil with added calcium carbonate (500 g) and magnesium (10 g) and 3) Cerr. - Savannah soil. After homogenization, the substrates were sent for analysis in the Chemical Analysis Laboratory of Soil and Plant, Department of Natural Resources, Agricultural Science Center - UFSCar, *Campus* Araras (Table 1). Phosphorus, potassium, calcium and magnesium extraction was performed with ionic exchange resin, and for determination of aluminium and potential acidity (H + Al) extraction was performed with KCl 1 mol L^{-1}, as described in EMBRAPA (2009).

Collection of samples and delimitations

After 37 days of growth in polyethylene bags commenced the beginning of the monthly collections of the experiment, and this process ended after growth of 230 days in a greenhouse. The experiment was conducted in a randomized block design in a factorial design 2 × 3 (two light intensities and three types of substrate) and each treatment (six) consisted of four plants, analysed monthly.

Mass determination and chemical analysis

The material was collected, packed in paper bags, and then placed in drying ovens with forced air circulation at 80 ± 5°C until constant weight and evaluated on analytical balance in order to determine the dry mass (leaves and total). Subsequently, the material was ground in a knife mill and subjected to chemical analysis, according to EMBRAPA (2009).

Interactions

In the unfolding of the interactions, growth days were considered within each luminous intensity for each substrate by adjusting the linear regression models in relation to growth time and analysing the angular coefficients of the adjusted linear equation as the rate of increase in the studied period.

Principal component analysis and statistics

The analysis of components is a multivariate statistical technique, based on the 1st main component (MC1) and 2nd main component (MC2); MC1 is defined as the maximum variability of the data explained and MC2, the maximum unexplained variability. It can also be said that MC1 and MC2 performs the analysis of the variables with the explained variability, placing them within grouping. Statistical analysis was performed using procedures PROC GLM, PROC REG and PROC PRINCOMP using SAS package (version 8.2).

RESULTS AND DISCUSSION

The substrates, light intensity and time of cultivation had a significant effect (p<0.01), either individually or through interactions, nutrient accumulation, dry matter in leaves and total dry matter (Tables 2, 3 and 4), indicating that the species *T. aurea* responded positively to different treatments and showed different patterns of development, depending on the assessed factor. According to Larcher (2003) and Lambers et al. (2008) it is expected that different environmental conditions will result in differentiated plant growth. In nutrient accumulation in the leaves (Table 2), all adjusted models were significant, with the exception of magnesium and potassium in the substrate Cerr., with luminous intensity of 950 µmol m^{-2} s^{-1}, indicating that the species had a different growth pattern, depending on the type of substrate or light intensity. Thus, their behaviour during growth is associated with the environmental parameter to which it is subjected. Since there exist different results for these two elements, substrate Cerr., could be related to its low availability in the soil (Table 1) and a greater need for absorption of these elements by the plant in the presence of higher brightness.

In relation to the intensity absorption of nutrients, the Ca-Mg and Cerr. substrates showed few elements being affected by a greater or lesser amount of light present (Ca-Mg substrate, nitrogen; Cerr. substrate, calcium). In relation to the OM substrate, a larger number of elements have its absorption intensity increased, such as sulfur, phosphorus and potassium, most probably related to the factor of availability of some elements of the substrate and their interaction when in the presence of intense light.

It is noteworthy that although almost all the elements have had their absorption intensity increased when the plants were kept in 950 µmol m^{-2} s^{-1}, magnesium reduces the intensity. Castro et al. (2001), working with six species of tropical forage grasses (*Andropogon gayanus*, *Brachiaria brizantha*, *B. decumbens*, *Melinis minutiflora*, *Panicum maximum* and *Setaria sphacelata*) indicated

Table 2. Nutrients accumulation (mg kg^{-1}) in *Tabebuia aurea* leaves in two light intensities (750 and 950 µmol m^{-2} s^{-1}) and three types of soil (Cerr. savannah soil; Ca-Mg - savannah with calcium carbonate and magnesium; OM – savannah with organic matter).

Parameter	Days	Ca		S		Mg		N		P		K	
		750	950	750	950	750	950	750	950	750	950	750	950
Ca-Mg	30	28.1	18.7	0.9	0.4	9.1	8.5	8.6	5.7	0.7	0.4	5.6	3.1
	60	28.9	12.3	2.7	1.4	6.5	4.8	28.2	13.1	2.0	0.7	17.0	7.9
	90	29.6	25.2	5.3	5.3	8.2	8.6	24.1	24.3	3.8	1.7	38.6	22.0
	120	57.9	55.5	9.4	15.9	16.5	26.0	46.8	85.9	6.9	4.3	41.9	62.3
	150	57.3	55.0	13.3	19.0	16.0	23.5	45.2	79.0	7.8	4.5	41.2	64.5
	180	104.2	83.3	21.2	28.9	24.4	27.6	67.0	94.1	10.4	5.7	57.1	70.6
	210	138.4	114.0	21.8	33.3	26.8	31.6	66.1	88.9	11.2	5.9	48.6	52.6
	230	124.5	140.9	23.7	36.9	26.1	33.6	69.7	110.1	11.6	7.4	48.4	47.7
Ac		0.54ª	0.50ª	0.54ª	0.5ª	0.12ª	0.15ª	0.33ᵇ	0.48ª	0.05ª	0.03ª	0.27ª	0.30ª
R^2		0.86**	0.90**	0.87**	0.87**	0.88**	0.86**	0.92**	0.85**	0.97**	0.92**	0.75*	0.58*
Cerr.	30	5.0	11.1	0.4	0.5	2.0	5.6	5.0	8.4	0.3	0.6	2.4	4.5
	60	15.5	19.5	1.2	1.1	4.4	5.9	14.8	12.7	0.9	0.9	7.8	9.8
	90	26.7	22.0	2.6	4.1	6.9	3.4	19.1	17.2	1.2	1.4	11.8	12.9
	120	27.0	57.6	4.6	6.1	7.1	6.6	21.3	28.7	0.7	2.5	9.9	15.5
	150	35.9	51.5	8.2	7.3	12.6	6.3	37.5	33.2	1.7	2.9	22.0	19.7
	180	35.3	79.5	10.5	9.2	12.6	6.5	39.3	37.5	2.0	3.1	22.7	20.4
	210	35.5	105.4	10.7	9.5	11.5	8.5	32.8	41.8	2.1	2.8	17.5	16.4
	230	56.1	111.3	14.1	11.1	15.2	10.1	49.0	46.7	2.9	3.2	22.7	13.8
Ac		0.22ᵇ	0.45ª	0.05ª	0.05ª	0.07ª	0.04ª	0.20ª	0.21ª	0.22ª	0.20ª	0.11a	0.10ª
R^2		0.90**	0.80**	0.99**	0.96**	0.90**	ns	0.86**	0.80**	0.86**	0.85**	0.78**	ns
OM	30	12.1	25.6	0.5	0.9	5.3	8.8	7.2	8.6	0.5	0.8	3.6	7.5
	60	46.7	54.1	2.5	4.9	8.2	11.2	31.2	36.1	1.8	4.7	18.9	35.0
	90	105.1	80.0	12.5	13.4	19.4	22.5	68.7	56.5	5.7	8.7	51.0	71.0
	120	190.7	307.0	23.4	50.5	27.4	71.2	123.5	196.9	9.7	31.1	68.1	226.7
	150	428.0	326.9	38.2	46.3	51.8	74.9	184.9	224.1	14.5	31.4	66.1	150.0
	180	403.2	388.5	50.5	78.3	57.3	100.5	150.2	205.1	17.8	41.9	73.6	157.4
	210	404.1	450.6	52.8	86.0	52.9	125.5	175.2	224.0	12.5	42.3	79.3	203.0
Ac		2.26ª	2.24ª	0.25ᵇ	0.39ª	2.27ª	0.51ᵇ	0.88ª	1.18ª	0.07ᵇ	0.22ª	0.37ᵇ	0.88ª
R^2		0.84**	0.89**	0.90**	0.88**	0.84*	0.90**	0.88**	0.86**	0.80**	0.89**	0.62*	0.54*

** and * = significant 1 and 5 %, respectively and ns= no significant. Ac = angular coefficient. Equal letters in the angular coefficients on the line for each nutrient, do not differ each other by Tukey test at 5% of probability.

that higher concentrations of magnesium occurred in greater shading, with high values for the leaves. This is due to the higher concentration of chlorophyll in the shaded plants and their constituents such as magnesium. In this manner, a higher luminous intensity could mean a lower uptake rate of this element, which could have occurred to *T. aurea*. Also, some studies indicate that plants in shady environments typically allocate higher amount of biomass in their leaves (Poorter et al., 2012), this could also mean a higher concentration of nutrients, which was not observed for most of the elements evaluated. This is probably related to the fact that their leaves are relatively thin and presence of low leaf mass density (Lambers et al., 2008).

In relation to increasing rates, the element phosphorus, substrate Ca-Mg, sulfur and magnesium elements, substrate Cerr., both luminosities and phosphorus element, substrate OM, 750 µmol m^{-2} s^{-1}, between nutrients studied presented the lowest increase rates, i.e they accumulated lesser amount in the evaluated structures (Table 2). In relation to sulfur, Ca-Mg substrate allowed greater rate of absorption of this element. Sulfur

Table 3. Dry matter accumulation rate (g/plant) in the leaves of *Tabebuia aurea* in two light intensities (750 and 950 µmol m^{-2} s^{-1}) and three types of soil (Cerr. savannah soil; Ca-Mg - savannah with calcium carbonate and magnesium; OM – savannah with organic matter).

Days	Ca-Mg		Cerr.		OM	
	750	950	750	950	750	950
30	0.33	0.38	0.22	0.45	0.49	0.37
60	1.01	0.88	0.69	0.71	2.05	1.88
90	1.52	1.65	0.89	1.00	4.05	3.63
120	2.76	4.83	0.91	1.65	7.82	12.95
150	2.90	5.00	1.91	1.91	11.27	13.61
180	4.35	5.74	2.03	2.04	13.65	16.75
210	4.47	5.85	2.05	2.35	13.91	18.43
230	4.84	6.71	2.53	2.65	14.82	19.00
Ac	0.021^{b}	0.030^{b}	0.011^{c}	0.012^{c}	0.067^{a}	0.086^{a}
R^2	0.96^{**}	0.90^{**}	0.94^{**}	0.97^{**}	0.93^{**}	0.89^{**}

** and * = significant 1% and 5%, respectively. Ac = angular coefficient. Equal letters in the angular coefficients on the line for each nutrient, do not differ each other by Tukey test at 5% of probability.

when in soils rich in organic matter is usually fixed, making it less available to plants. However according to Crusciol et al. (2014), application of plaster doses may cause increase in $S\text{-}SO_4$ content in the soil, which is reflected in the increase in the concentration in leaves, a similar result obtained for *T. aurea*. Thus, soils with the presence of some form of calcium could facilitate the absorption and accumulation of $S\text{-}SO_4$.

Cerr. substrate has, among substrates tested, the lowest concentration of Mg, which could explain the lower rate of increase in this substrate. Already phosphorus exists in small quantities in Cerr. and Ca-Mg substrates, but the growth rate was lower only in Ca-Mg substrate. This substrate by adding calcium and magnesium affected the availability of the element for plants of *Tabebuia*, an effect explained by calcium and phosphate reaction to form compounds of low solubility, a process known as precipitation. However according to Tokura et al. (2011), studying the dynamics of phosphorus in soil after the addition of limestone (up to 150 days), in some situations this liming may not cause a reduction in the activity of the element in the soil, a factor related to other existing environmental conditions.

In this study, the period was 230 days, with the use of soluble source of calcium and magnesium. Therefore, there was no time and availability of PO_4^{-3} and Ca^{+2} for the phenomenon known as retrogradation, as discussed in Novais et al. (2007). This process may also be responsible for the reduction in phosphorus accumulation rate, with increasing light intensity, and hence, the greater the soil mass flow by evaporation process. Mengel and Kirkby (2001) and Marschner (2012) noted that calcium movement into the soil is basically through mass flow and phosphorus is by diffusion; thus, the higher the mass flow, the more calcium in the soil solution, and the greater the reaction with phosphorus. Another factor that enhances the interaction between calcium and phosphorus is that at the end of the study period, between chemical elements, calcium was the most accumulated, and phosphorus the least accumulated in the dry mass of leaves, regardless of the substrate and the light intensity. In Cerr. substrate, all the nutrients studied, with the exception of phosphorus, showed lower accumulation rate values, when compared to Ca-Mg and OM, or the two light intensities. These data also show that *T. aurea* species is adapted to environments with low phosphorus availability, with ability to absorb calcium; this also indicates that it is a calcicole species, common in calcareous soil. Crusciol et al. (2014) reported that some plant species develop mechanisms to increase the efficiency in the absorption of calcium by increasing the root system and a high absorption rate of root length unit, thereby achieving high inflow of this nutrient.

According to Soares and Oliveira (2009), *T. aurea* species develops in large areas known as ‘paratudais’, with the predominance of only one tree species dominating the landscape in the Pantanal of Miranda, Mato Grosso do Sul. These areas belong to Miranda River floodplain, whose waters are rich in carbonate compounds, giving the soil of the region high levels of calcium and magnesium.

Light intensity affects the amount of organic matter accumulation in the leaves (Table 3), with increasing light intensity leading to increased nutrient accumulation rate on the dry weight of the leaves. OM substrate, 950 µmol m^{-2} s^{-1}, showed the best performance, followed by Ca-Mg, 950 µmol m^{-2} s^{-1}, indicating that the improved availability of nutrients in OM substrate is essential in expressing the intensity of light as a factor of better development of the plant.

Scalon et al. (2006a), working with *Enterolobium contortisiliqunn* (Vell.) Morong, found higher dry mass in plants with higher brightness, similar to the results found in this study, showing different growth strategies, a factor related to the environment in which species develop. According to Epstein and Bloom (2006) and Lambers et al. (2008), this is a pattern often found in nature. The second best results found for the Ca-Mg substrate, despite differences in concentration of nutrients on substrates (Table 1), also indicates the species studied as calcicole grows even on poor soils, but with a high concentration of calcium and magnesium, allowing its good growth in this environment. The higher pH of this substrate is also a factor that provided better development for the seedlings grown in this environment, because according to Lambers et al. (2008), the growth of calcicole species is better on soils with a high pH.

Table 4. Total dry matter rate (g/plant) of *Tabebuia aurea* in two light intensities (750 and 950 µmol m^{-2} s^{-1}) and three types of substrates (Cerr. pure Cerrado soil; Ca-Mg - Cerrado soil with addition of calcium carbonate and magnesium; OM – Cerrado soil with addition of organic matter).

Days	Ca-Mg		Cerr.		OM	
	750	**950**	**750**	**950**	**750**	**950**
30	0.71	0.78	0.53	1.05	0.94	0.81
60	2.45	2.12	1.76	1.88	4.26	3.97
90	3.29	4.22	3.01	3.30	8.73	9.22
120	6.61	12.12	3.78	5.23	16.70	30.56
150	7.83	14.65	6.16	7.14	36.98	43.25
180	13.33	26.56	6.97	9.75	48.38	81.10
210	20.16	32.10	9.52	15.79	59.85	101.05
230	22.18	63.28	12.51	21.38	91.95	129.73
Ac	0.079^{d}	0.17^{c}	0.044^{d}	0.068^{d}	0.285^{b}	0.43^{a}
R^2	0.83^{**}	0.67^{*}	0.90^{**}	0.79^{**}	0.78^{**}	0.79^{**}

** e * = significant 1% and 5%, respectively. Ac = angular coefficient. Equal letters in the angular coefficients on the line for each nutrient, do not differ each other by Tukey test at 5% of probability.

According to Soares and Oliveira (2009), *T. aurea* is a specie calcicole or an important tree of areas with soil containing high levels of calcium and magnesium.

In relation to nitrogen, substrate Ca-Mg, calcium element, substrate Cerr. and nutrient potassium, substrate OM, there was an increase of respectively, 58.8, 98 and 155.9% in the quantity of elements on the dry weight of the leaves, after 230 days. This increase in dry weight is associated with increase in light intensity from 750 to 950 µmol m^{-2} s^{-1}. The nutrient uptake by plants occurs by passive and active processes, being active with energy expenditure, i.e, requiring adenosine triphosphate (Larcher, 2003; Lambers et al., 2008; Taiz et al., 2014). Thus, the plant in greater light intensity, therefore, with greater production of photo assimilates, once the nutrients are available, favours higher rate of accumulation of organic matter, as observed in OM and Ca-Mg substrates (Tables 2, 3 and 4), corroborating the data of Aguiar et al. (2011), who worked with the shading effect of the species *Caesalpinia echinata* Lam.

In the dry matter accumulation rate of leaves, OM substrate, there was no effect of intensity (Table 3), despite increase in value of light intensity to 950 µmol m^{-2} s^{-1}. These data indicate that the luminous intensity increases the rate of formation of organic compounds, but not the formation of dry matter, suggesting that the increase was more of an internal than external factor, i.e., balancing the needs of the plant. This fact is reinforced when analysing the total matter, where OM substrate, the rate of total dry matter was influenced by luminosity (Table 4), indicating effect on the formation of organic matter in the shoot and possible accumulation of ions (nutrients) in the roots. The analysis of the dry weight of the root system at the end of period of 230 days indicated that the OM substrate accumulated a larger amount of resources, totaliing 77.84 and 111.3 g pl^{-1} (750 and 950 µmol m^{-2} s^{-1}), while the Ca-Mg substrate, 17.71 and 57.43 g pl^{-1} (750 and 950 µmol m^{-2} s^{-1}) and Cerr., 10.46 and 19.03 g pl^{-1} (750 and 950 µmol m^{-2} s^{-1}) statistical result was well established and discussed, which demonstrates the negative effect of higher shading.

Data from Scalon et al. (2006b), working with seedlings of *Schinus terebinthifolius* Raddi, also found that the shading increased the total dry matter and reduced the specific weight of the leaves. This lighting effect may be related to the transpiration of plants, as found by Dutra et al. (2012), where it was verified that shading reduced perspiration of seedlings of *Copaifera langsdorffii* Desf.

The data obtained for *T. aurea* indicate an increase in mass production of roots due to luminosity, contradicting the data from Cavalcante et al. (2011), who worked with *Hylocereus undatus* [Haw.] specie Britton & Rose, and verified that the increase of the shadowing causes increased dry weight of root. However, this increase in root mass does not reflect significantly in the dry matter of the leaves, suggesting that this species has an ion storage mechanism in the root system. Oliveira and Gualtieri (2011, 2012) and Oliveira and Perez (2012) results indicated that the species has, during the course of its development a tendency of higher photo assimilates accumulation in the root system. According to Oliveira and Gualtieri (2012), the largest accumulation of dry biomass in the root system of *T. aurea* could be a survival strategy during bad times. This allocation provides higher reserves when environmental conditions, such as drought stress, which often occur in the region of origin of species, are adverse. In this manner, the plants grown with more light would be tailored for a possible environmental stress.

With regard to the principal component analysis, this allowed the realization of the analysis variables with the variability explained by placing them within clusters (Figure 1). In the assessment of the chemical element potassium, this was the most varied with the environment. Thus, the reduced availability of nutrients in Cerr. substrate in the luminosity 750 µmol m^{-2} s^{-1}; according to the analysis, the potassium was within the same group of other variables analysed, indicating stressful environment for plants, due to the fact that the plant absorbed what was only necessary for survival.

Since environmental stress conditions have decreased due to the increase amount of potassium in the substrate, the element was separated from the other variables, forming another group and does not correlate with other nutrients, dry matter and organic matter, showing a consumption luxury, it would be the ability to absorb more potassium than their needs. This mineral nutrient has no structural function in plant metabolism, but it is the most abundant cation in the cytoplasm and is highly mobile in the plant, which greatly contributes to the maintenance of osmotic potential of cells and tissues, acting as enzyme activator and as neutralizing anionic macromolecules (Epstein and Bloom, 2006; Marschner, 2012).

Another factor to consider is that the species in its habitat, Pantanal of Miranda, grows in soils with high content of calcium and magnesium, which exhibit competitive effect with potassium, thus becoming a species that absorbs little potassium. Another fact to explain the difference in absorption would be the root system, where during initial growth, the species accumulated more dry mass of root, so that an increased demand for calcium and magnesium was seen in pectate formation which composed the middle lamella of the plasma membrane (Taiz and Zeiger, 2010; Taiz et al., 2014). The level of potassium in the leaves have a distinct pattern when compared with calcium, which with the passage of time increases concentrations of leaves and magnesium, which remains; the potassium initially increases its concentration and then, or tend to stabilize or decrease its concentration (Table 2), depending on the treatment. On the other hand, for this element, shaded plants also have higher nutrient levels in leaves. As opposed to the other elements, treatments with OM presented at the end of the experiment, the highest concentrations of potassium in leaves, indicating their importance for the growth of seedlings. This fact should be directly related to the concentration of potassium in the substrate, which was five times more in the treatment OM. To this nutrient, the results obtained seem to indicate that the dry weight has a highest correlated value in the OM treatment with higher potassium concentration, as opposed to magnesium and calcium.

Mengel and Kirkby (2001) and Marschner (2012) wrote that high concentrations of calcium and magnesium reduced the absorption of potassium element. However, this was not observed for the leaves of this species, particularly for the treatment with Ca-Mg. They also underscored that occurring in the lower shaded plants were rates of absorption and dry root weight (the inhibition of absorption of potassium in low light intensities is caused by DNP 2,4- - inhibitor of mitochondrial phosphorylation – formation of ATP). However, to the species *T. aurea,* this process was not observed when subjected to different light intensities, indicating its adaptation to different lighting conditions, with the highest concentration of the element in shaded plants. In accordance with Oliveira and Gualtieri (2012), *T. aurea* has a high phenotypic plasticity to different environmental conditions.

According to Mengel and Kirkby (2001) and Marschner (2012), with the increasing age of the leaves, there is a decrease in the concentration of this element in the leaves of many plant species, this behavior is exhibited by *T. aurea*. The decreasing trend in the concentration of this mineral in the leaves was more rapid when using soil with addition of organic matter and Ca-Mg and less in Cerrado soil probably because in this case the issuance of new leaves is much slower and, consequently, the decrease of potassium in the leaves is smaller, since they take longer time to emerge. In a study, Marschner (2012) cited that older acicula of *Picea abies* (L.) H. Karst. showed lower potassium concentrations, demonstrating that over time, there is a natural reduction in nutrient concentration in leaves.

By comparing the current levels of macronutrients in *T. aurea* leaves with appropriate concentrations found in cultivated species of forest trees (pines, araucaria, eucalyptus and rubber trees), it can be seen that the use of fertilized soils with organic matter afforded average values suitable for the normal development of forest species and probably suitable for the development of this tree species. However, in the soil without fertilization, the foliar values of this element are at the lower limit of what can be considered suitable for adequate growth of those species. Already, the soil with the addition of Ca-Mg propitiated foliar concentration of this nutrient that is below the levels required for the best growth of the mentioned species. Nevertheless, *T. aurea* may properly develop this type of substrate, a factor probably related to the presence of calcium and magnesium.

In potassium deficient plants, significant chemical changes occur because potassium is a constituent or activator of several enzymes, among other actions (Taiz and Zeiger, 2010; Taiz et al., 2014). Photosynthesis is also affected by potassium because an increased concentration of this element stimulates CO_2 fixation (decreasing the diffusive resistance for CO_2 in the mesophyll), and again promotes the synthesis of the enzyme ribulose bisphosphate carboxylase in addition to other effects, as in cell elongation. In this manner, plants with this deficiency experience late growth (Mengel and Kirkby, 2001; Marschner, 2012).

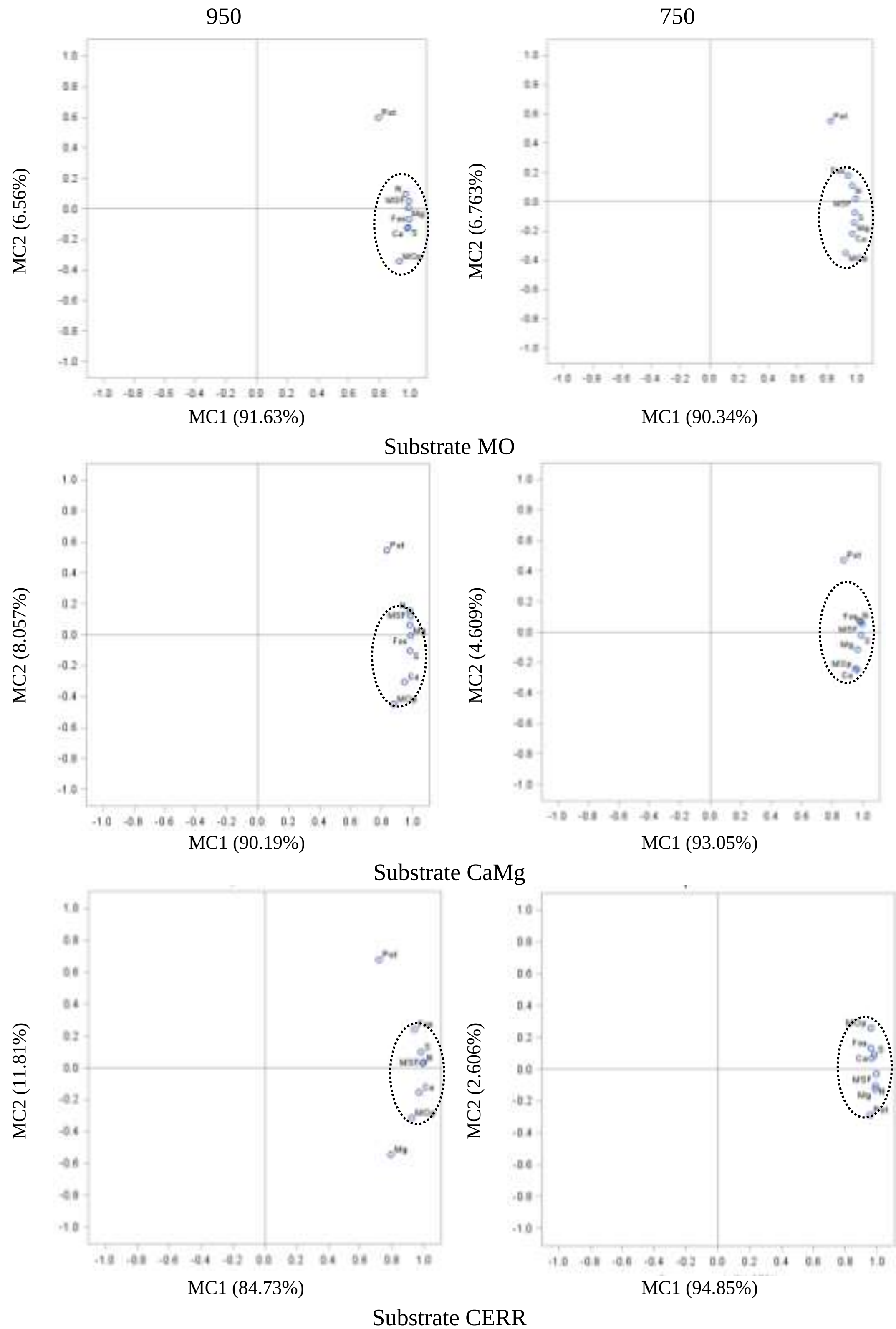

Figure 1. Principal component analysis of macronutrients, dry matter and organic of aerial part, for substrates and luminosity, *Tabebuia aurea*.

Although plants grown in soils with addition of Ca-Mg showed similar potassium values in the substrate, compared to those grown in Cerrado soil, its development was higher with higher dry matter accumulation in leaves and total, indicating that the available soil potassium was suitable for both and the difference in growth should be associated with a higher amount of calcium and magnesium in the treatment with Ca-Mg. Nevertheless, the best performance was observed for plants grown in soil with added organic matter.

Conclusions

The *T. aurea* species has characteristics of calcicoles species and better development when grown in brighter locations. The addition of organic matter provided the best development, a situation related to their higher fertility; but when cultivated in poorer soils, the presence of calcium and magnesium favours its development.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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Full Length Research Paper

Population fluctuation and infestation of *Drosophila suzukii* in berry crops in Southern Brazil

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Drosophila suzukii **is an insect pest with high capacity to cause damage to soft-skinned fruit whose behavior on host crops in Brazil is still unknown. This study aims to determine the population fluctuation of *D. suzukii* on different berry crops from 2014 to 2017 and infestation. In each orchard, two traps containing attractant (biological yeast + sugar + water) were installed each week; and sorting was done in laboratory after two days hold-up period. At the same time, fruits were harvested to assess the presence of *D. suzukii.* The emergence of *D. suzukii* was observed in December (late spring), population increased from January to March (summer) and terminated in June (late fall). In the winter, there were no adults in the traps. The crop of blackberry was highly preferred in first two years, followed by raspberry and strawberry. In the third year, the preference was for raspberry. The blueberry is less attacked due to the asynchronization between the fruiting time and occurrence of the pest.**

Key words: Spotted wing drosophila, blackberry, raspberry, blueberry, strawberry.

INTRODUCTION

Drosophila suzukii (Matsumura, 1931) (Diptera, Drosophilidae) is a polyphagous quarantine pest (Cha et al., 2014) with elevated dispersion capacity, which cause damage in developing fruits. The registered species in 2008 was in several places in the United States, Canada and EU; they bring about elevated damage to various fruit crops (Hauser, 2011). In Brazil, the first registered occurrence was made at the Vacaria City Region, at Rio Grande do Sul State, (In Southern Brazil), with 30% of damaged fruits of strawberry (Santos, 2014). Worldwide, the insect pest is known as “Spotted Wing Drosophila (SWD)”, but in Brazil, the term “Suzuki” is used as the insect’s common name.

This insect pest has a wide range of hosts, and attacks different fruit species including berry crops (Bolda et al., 2010; Goodhue et al., 2011). There are several records of significant damage in cherry, blackberry, raspberry, strawberry and blueberry (Lee et al., 2011; Walsh et al., 2011). In contrast to other *Drosophila* species, which prefer damaged fruits for oviposition, *D. suzukii* uses ripe

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fruits and its sclerotized serrated ovipositor is capable of puncturing the skin of straight fruits for oviposition (Lee et al., 2012; Wallingford et al., 2015). Burrack et al. (2017) reported that in USA, the insect pest has a pattern of preference for some crops and the synchrony between fruiting and the presence of the insect pest is a key factor for the management of *D. suzukii*. As the pest was identified in Brazil at only four years, there is a lack of information about the population aspects on host crops as well as the resulting damage. These aspects limit the preparation of control and management practices of this species in Brazil. Thus, the objective of this study is to determine the population fluctuation of *D. suzukii* on the crops of blackberry, raspberry, strawberry and blueberry to correlate the occurrence with fruit infestation with eggs.

MATERIALS AND METHODS

The study was carried out in a commercial orchard with diverse berries production, located at the Vacaria city region, Rio Grande do Sul State, Brazil (28°28'41.95"S and 50°58'11.74"W) between August of 2014 and December of 2017. The assessed crops were blackberry (variety 'Tupy' and 'Thompson'), and raspberry (variety 'Heritage') both crops grown in the soil, under plastic high tunnel cover; strawberry (variety 'Albion') grown into greenhouse and cropped on substrate, and blueberry (variety 'O'Neal' and 'Duke') grown under hail nets (Figure 1). For the study, each crop was considered as an experimental unit.

In each crop, two traps were installed for monitoring. It was made from colorless Coca Cola™ plastic bottles (250-ml) previously washed with tap water and dried which contained five holes of 0.5 cm diameter; they were filled with a yeast based *Sacharomyces* spp., sugar and water as suggested by Santos (2016), for monitoring adults of *D. suzukii* (Figure 2).

Traps were changed at weekly interval and placed at the intermediate position of the plant's canopy (blackberry, raspberry and blueberry) and above the plants on strawberry, keeping for two days hold up period. They were gathered for assessment at the Embrapa Uva and Vinho Entomologic Lab facilities, using a stereomicroscope with a 2-fold enhancement for insect identification and counting. At the time of trap collection, a sample of 15 fruits from each experimental unit was picked up randomly for the assessment of *D. suzukii* oviposition.

The data were tabulated and the total average of adults trapped per day in each trap was calculated (symbolized in Brazil by MAD) from each monitoring trap. A pest population curve for each fruit crop was prepared throughout the experiment. The sex ratio was assessed using the total number of *D. suzukii* trapped during the study, in each crop, and subjected to the chi-square (χ^2) test at 5% of probability. MAD and the percentage of eggs found in the fruits were used to determine the difference among crops and years. The data were subjected to analysis of variance and the means were sorted by the Tukey test at 5% of probability.

RESULTS AND DISCUSSION

The total number of *D. suzukii* trapped in all sites was 8553 (4304 males and 4249 females) with no significant difference on the sexual proportion (χ2= 0.354; DF = 1; p = 0.5593), during the 177 evaluations.

In general, female *D. suzukii* adults was seen constantly at late spring (November 2014), males being detected from December of that year. Trapping continued until June 2015. During the winter the pest was not detected; a new incidence occurred only during the following late spring (December 2015), following the same pattern as previous year, with trapping stopped in late October. The same behaviour was found in the subsequent years of the study (Figure 3).

On the blackberry crop, throughout the experiment, 2724 individuals were entrapped (1346 males and 1378 females) without any significant difference between males and females (χ^2 = 0.376; DF = 1; p = 0.5525).

The presence of *D. suzukii* was first observed on September 22nd, 2014 when an insect was trapped at the experimental unit without the presence of developing fruits; but this did not happen in the subsequent years. The constant occurrence of the pest and elevated MAD at the site were registered from November 24th, 2014. The greater peak of females occurred in February 2nd, 2015 with 13 MAD, while for males, later, on April 13th, 2015 with 29.25 MAD. Adult entrapped individuals were registered until June 2015; they restarted activity in December 2015 until late of May 2016. The same pattern was repeated in 2017 (Figure 4, Appendix Table 1).

On the raspberry crop, 4376 individuals were trapped (2337 males and 2039 females) with significant difference on the sexual proportion (χ^2 = 20,293; DF = 1; p = 0.0001).

The trapping started in early December 2014 with peaks of males of 24.25 MAD in January 1st, 2015 and females of 8 MAD in March 2nd, 2015, ending the entrapments in June 2015. The presence of the insect pest was noticed again between December 2015 and May 2016 with the same pattern found in 2017 (Figure 5, Appendix Table 2).

On the strawberry crop, 1037 individuals (506 males and 531 females) were trapped, without significant difference of the sexual proportion (χ^2 = 83,163; DF = 1; p = 0.0001).

The trapping of *D. suzukii* on the crop started in December 2014 although with decreased number of adults trapped, compared to blackberry and strawberry. The greatest records occurred in March 16th, 2015 when 4.25 males and 3.50 females (MAD) were trapped. The end occurred in April 2015. In the following years the fluctuations of *D. suzukii* followed the same pattern, but with elevation of the population in the year 2017 (Figure 6, Appendix Table 3).

On the blueberry crop, 416 individuals were trapped (115 males and 301 females) with significant difference on the sexual proportion (χ^2 = 37,59; DF = 1; p = 0.0001). The population of *D. suzukii* remained below the level found for the other crops studied. The trapping started in December 2014 and ended in January 2015; it resumed in December 2015, when there was greater occurrence in the crop with 15.75 MAD of females in December 28th, 2015. In 2016 and 2017, the pattern of occurrence was

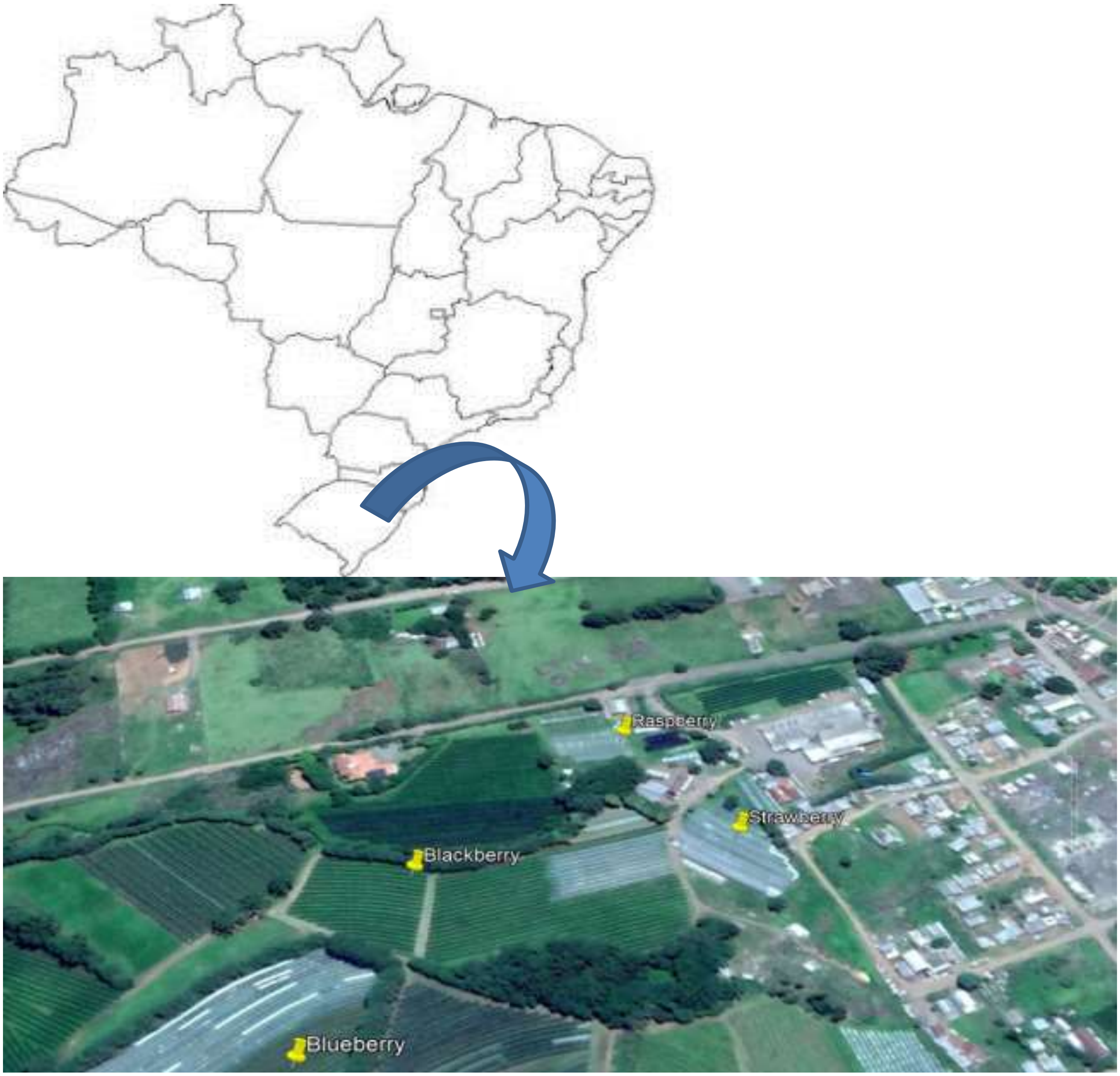

Figure 1. Location of experimental units: Blackberry, Raspberry, Strawberry and Blueberry. Vacaria, Rio Grande do Sul, Brazil.

Figure 2. (A) Picture of the trap, (B) Detail of hole.

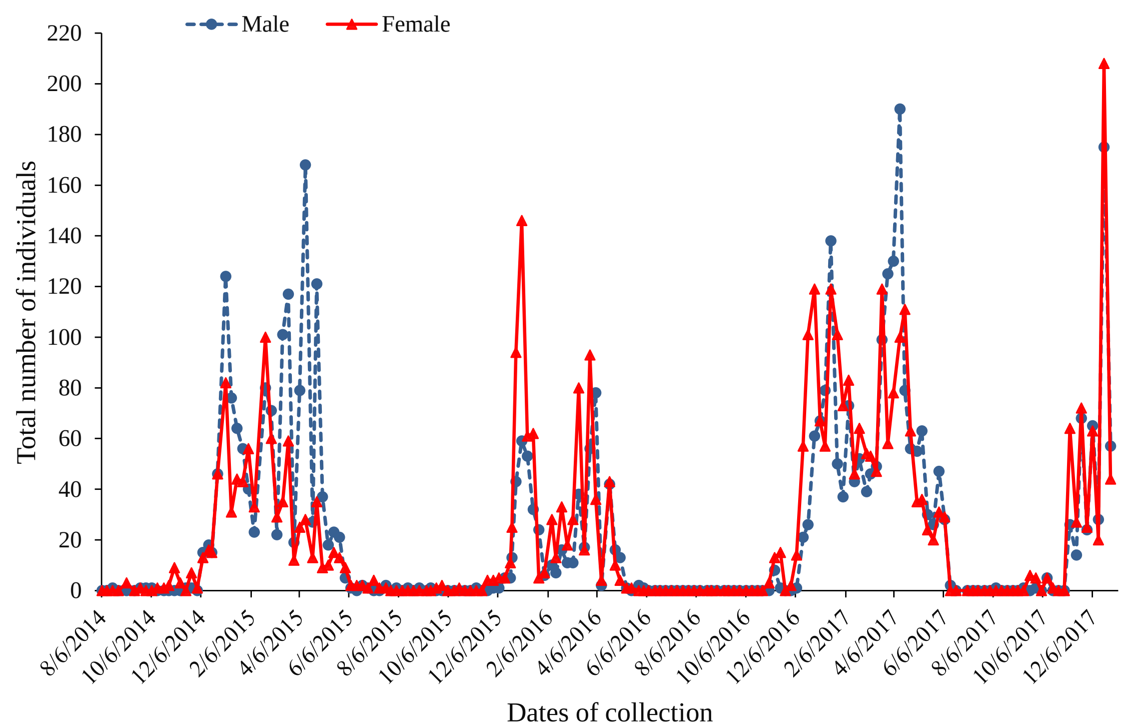

Figure 3. *Drosophila suzukii* adults (male and female) population fluctuation in orchards of blackberry, raspberry, strawberry and blueberry from Vacaria, RS, Brazil in 2014/2017.

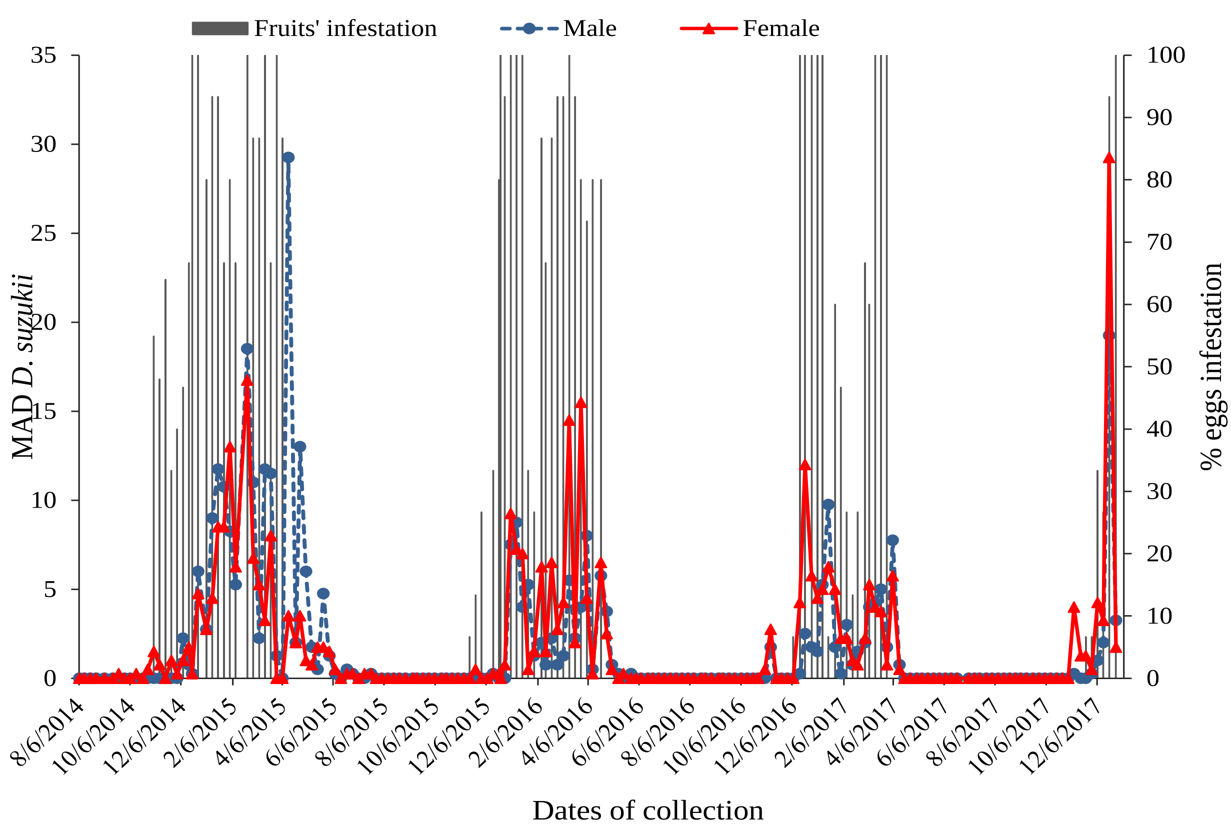

Figure 4. *Drosophila suzukii* adults' population fluctuation in orchards of blackberry from Vacaria, RS, Brazil in 2014/2017 (Appendix Table 1).

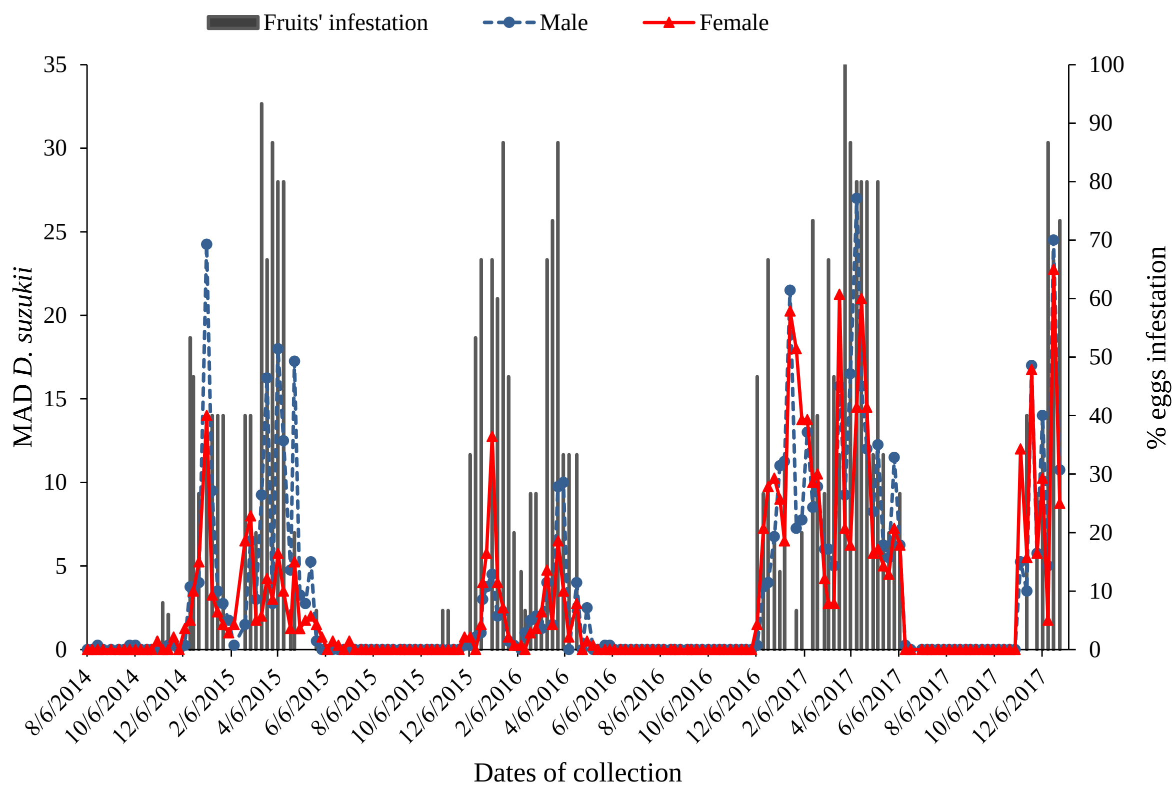

Figure 5. *Drosophila suzukii* adults' population fluctuation in orchards of raspberry from Vacaria, RS, Brazil in 2014/2017 (Appendix Table 2).

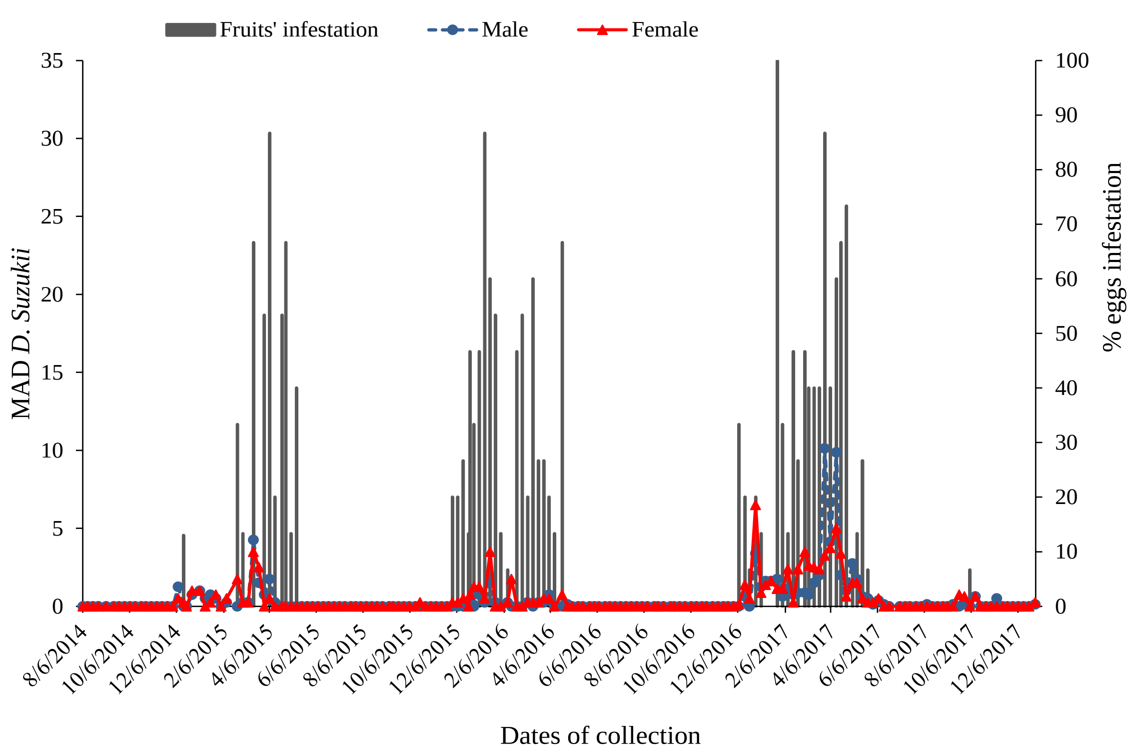

Figure 6. *Drosophila suzukii* adults' population fluctuation in orchards of strawberry from Vacaria, RS, Brazil in 2014/2017 (Appendix Table 3).

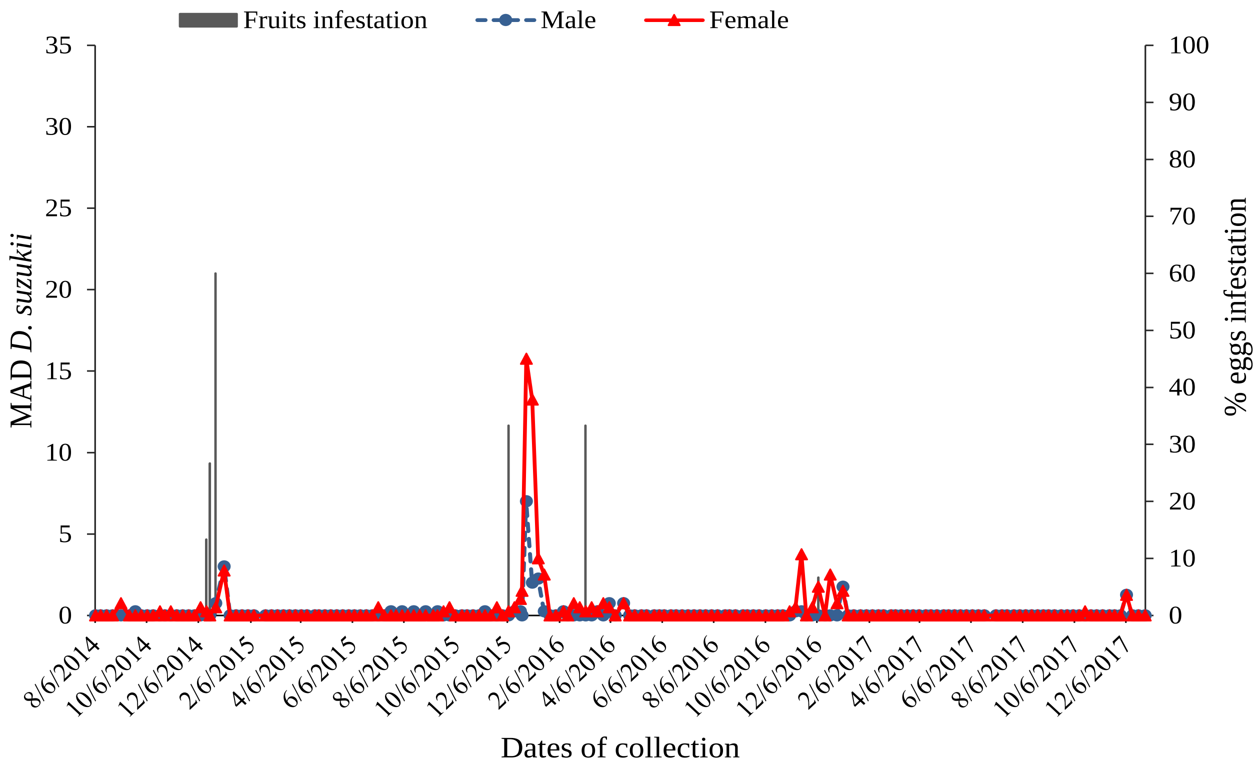

Figure 7. *Drosophila suzukii* adults' population fluctuation in orchards of blueberry from Vacaria, RS, Brazil in 2014/2017 (Appendix Table 4).

the same (Figure 7, Appendix Table 4).

On the assessed crops, blackberry, raspberry and strawberry, a similar trend in trap catches of *D. suzukii* start in early spring; was observed with higher numbers trapped throughout the summer, and then declined during winter, except blueberry, which started and ended during summer. The period of *D. suzukii* occurrence is in agreement with the records obtained from Europe and United Stated (Harris et al., 2014; Kinjo et al., 2014; Mazzetto et al., 2015; Aldea, 2015); it demonstrates a relation with temperature and availability of any food resource at the region. *D. suzukii* is a pest adapted to environments with mild temperatures at a range between 13.4 and 28.1ºC (Tochen et al., 2014). At the study's region, there is suitable temperature for the species as pointed out by Benito et al. (2016) as a critical area for the establishment of the pest in Brazil, confirmed by this study. Burrack et al. (2017) stated that the *D. suzukii* infestation risk is also associated to seasonality of fruit production and the preference of the pest for the host plants. In fact, the occurrence of adults in the traps was significantly greater on the raspberry crop in relation to strawberry and blueberry crops in the first and second years, and all crops in the third year of crop assessment (Table 1). On raspberry, it is possible to observe significant elevation on the occurrence of the pest in the third year of crop assessment (Table 1). Thus, the absence of *D. suzukii* during the winter may be related to the lack of host fruit crops apart from low temperatures. It is possible that during this period the insect pest could be in diapause, as related to other countries (Dalton et al., 2011; Walsh et al., 2011).

The fruit infestation with *D. suzukii* eggs tailed the adult's population fluctuation in all crops, and varied between 6 and 100% in blackberry, raspberry and strawberry. Blackberry presented the greatest number of sampling occasions (trap's gathering) with 100% of attacked fruits (16.7, 16.7 and 26.7%) on the first, second and third cropping seasons, respectively. On raspberry and strawberry, the totality of infested fruits with eggs was only seen once in the third year of study.

The mean infestation percentage in blackberry varied between 37.1 and 55.3% being statistically superior to the other crops in the first two years, and similar to what was found in raspberry and strawberry crops in the third year (Table 2). On raspberry, the percentage varied from 28.1 to 37.1% with significant elevation in the third year of study (Table 2). The same behaviour of significant elevation of fruit infestation was verified with the strawberry crop, where the percentages varied from 15.3 to 28.7%. On blueberry crop, the infestation was low and statistically similar in the three years of study (Table 2).

The period of crop's fruiting of the assessed crops at the study's region: blackberry (December to March),

Table 1. Mean adult insects (MAD) trapped (± standard error) of *Drosophila suzukii* trapped on the crops: blackberry, raspberry, strawberry and blueberry from Vacaria, RS, Brazil in 2014 to 2017.

Crop	Cropping season		
	2014/15	**2015/16**	**2016/17**
Blackberry	9.4±1.8^{Aa}	5.3±1.2^{ABa}	4.3±0.9^{Bb}
Raspberry	8.1±1.6^{Ba}	3.8±0.9^{Bab}	16.5±2.2^{Aa}
Strawberry	0.9±0.3^{Bb}	0.6±0.2^{Bc}	6.8±1.4^{Ab}
Blueberry	0.3±0.2^{Bb}	1.9±0.9^{Abc}	0.4±0.2^{Abc}

*Upper case letters compare cropping seasons in each crop (line), and lower case letters compare crops in each year (column) by the Tukey test at 5% of probability.

Table 2. Mean percentage of ovipositions (± standard error) of *Drosophila suzukii* in fruits of the crops: blackberry, raspberry, strawberry and blueberry from Vacaria, RS, Brazil in 2014 to 2017.

Crop	Cropping season		
	2014/15	**2015/16**	**2016/17**
Blackberry	55.3±7^{Aa}	54.9±7.5^{Aa}	37.1±7.9^{Aa}
Raspberry	28.1±5.5^{Bb}	28.4±5.3^{Bb}	37.1±5.8^{Aa}
Strawberry	15.3±4.6^{Bbc}	25.3±4.5^{ABb}	28.7±5^{Aa}
Blueberry	3.3±2.2^{Ac}	2.2±1.5^{Ac}	0.2±0.2^{Ab}

*Upper case letters compared to cropping seasons in each crop (line), and lower case letters compared to crops in each year (column) by the Tukey test at 5% of probability.

raspberry (December to May), blueberry (December to January) and strawberry (December to May) are linked to the period of incidence of the pest in the orchards. The staggered host fruit supply and the insect's preference guided the population dynamics at the studied area, where there is a clear dispersion among crops. Based on the collected populations and the eggs infestations, the pest preference for blackberry and raspberry in Southern Brazil is outstanding.

Although reports from overseas point out the preference of *D. suzukii* to raspberry crop, Aldea (2015) in Spain observed greater population and damage caused by the pest in raspberry, and little damage in strawberry. In the United States, Burrack et al. (2013) host preference tests were performed in laboratory with raspberry, blackberry, strawberry and blueberry where greater oviposition was found in raspberry and little oviposition in blueberry.

In the present study, strawberry seemed not to be the preferential host crop for *D. suzukii* when other host crops are available around the cropping site, although in places with exclusive strawberry production the losses might be significant, as reported by Santos (2014) in Vacaria, RS.

The lower infestation level at the blueberry crop in this study is related to the variety of fruit species grown closely in the same area, which results in low infestation of *D. suzukii*. The variety O'Neal (southern highbush) is considered as precocious at the study's region, and the ripening of fruits occurring until December, but for the variety Duke (highbush) classified as late-bearing variety produces until January (Pagot, 2006). The fact is its' fruiting period and harvesting happens when *D. suzukii* is with reduced populations and beginning of infestation, making these varieties to bypass the attack from the study's region. For other crops and places this aspect has been related; for example, strawberry in the United States reportedly has low *D. suzukii* damage due to asynchrony between fruit production and the presence of the pest (Burrack et al., 2017).

Conclusion

The occurrence of *D. suzukii* in Southern Brazil is related to the availability of yielding host fruits which start at late spring and end on late fall, without winter.

The fruit infestation with *D. suzukii* eggs tails the adults' population fluctuation on the host crops, and might reach 100%.

In the presence of many host crops cultivated closely in an area, the preferred crops by *D. suzukii* are blackberry and raspberry, followed by strawberry. Among the Blackberry and raspberry crops there was inversion of adults occurrence along the years, higher for blackberry in 2014/15 and 2015/16, and raspberry in 2016/17.

The attack on the blueberry crop is little in early and mid-bearing varieties due to lack of synchrony between the occurrence of the pest and fruit production.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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APPENDIX

Table 1. Mean adult insects (male and female) trapped per day (MAD) e percentage of eggs infestation (%) of *Drosophila suzukii* on the blackberry from Vacaria, RS, Brazil in 2014 to 2017.

2014				2015				2016				2017			
Data	**M**	**F**	**%**	**Data**	**M**	**F**	**%**	**Data**	**M**	**F**	**%**	**Data**	**M**	**F**	**%**
8/6	0	0	0	1/5	2.75	2.75	80	1/4	7.5	9.25	100	1/5	1.5	4.5	100
8/12	0	0	0	1/12	9	4.5	93.33	1/11	8.75	7.25	100	1/11	5.25	5	100
8/19	0	0	0	1/19	11.7	8.5	93.33	1/18	4	7	100	1/18	9.75	6.25	6.66
8/26	0	0	0	1/26	10.7	8.5	66.66	1/25	5.25	0.5	33.33	1/26	1.75	5	60
9/5	0	0	0	2/2	8.25	13	80	2/1	1.25	1.5	26.66	2/2	0.25	2.25	46.66
9/15	0	0	0	2/9	5.25	6.25	66.66	2/10	2	6.25	86.66	2/9	3	2.25	26.66
9/22	0	0.25	0	2/23	18.5	16.7	100	2/15	0.75	1.5	66.66	2/16	0.75	1	13.33
9/29	0	0	0	3/2	11	6.75	86.66	2/22	2.25	6.5	86.66	2/22	1.5	0.75	26.66
10/6	0	0	0	3/9	2.25	5.25	86.66	2/29	0.75	2.75	93.33	3/3	2	2.25	66.66
10/13	0	0.25	0	3/16	11.75	3.25	100	3/7	1.25	4.25	93.33	3/8	4	5.25	60
10/21	0	0	0	3/23	11.5	8	66.66	3/14	5.5	14.5	100	3/15	4.25	4	100
10/27	0	0.5	0	3/30	1.25	0	100	3/21	2.25	2	93.33	3/22	5	3.75	100
11/3	0	1.5	54.9	4/6	0	0	86.66	3/28	4	15.5	80	3/29	1.75	0.75	100
11/10	0	0.75	48	4/13	29.25	3.5	0	4/4	8	4.5	73.33	4/5	7.75	5.75	0
11/17	0	0	64	4/22	2	2	0	4/11	0.5	0.25	80	4/13	0.75	0.5	0
11/24	0	1	33.33	4/27	13	3.5	0	4/21	5.75	6.5	80	4/19	0	0	0
12/1	0	0.25	40	5/4	6	1	0	4/28	3.75	2.5	0	4/26	0	0	0
12/8	2.25	1	46.66	5/11	1.75	0.75	0	5/4	0.75	0.5	0	5/4	0	0	0
12/15	0.75	1.75	66.67	5/18	0.5	1.75	0	5/12	0.25	0	0	5/10	0	0	0
12/19	0.25	0.25	100	5/25	4.75	1.75	0	5/18	0	0.25	0	5/17	0	0	0
12/26	6	4.75	100	6/1	1.25	1.5	0	5/27	0.25	0	0	5/24	0	0	0
				6/8	0.25	0.5	0	6/2	0	0	0	5/31	0	0	0
				6/15	0	0	0	6/8	0	0	0	6/7	0	0	0
				6/22	0.5	0.25	0	6/16	0	0	0	6/14	0	0	0
				6/29	0.25	0.25	0	6/22	0	0	0	6/21	0	0	0
				7/6	0	0	0	6/29	0	0	0	7/5	0	0	0
				7/13	0	0.25	0	7/6	0	0	0	7/12	0	0	0
				7/21	0.25	0.25	0	7/13	0	0	0	7/18	0	0	0
				7/27	0	0	0	7/20	0	0	0	7/26	0	0	0
				8/3	0	0	0	7/27	0	0	0	8/2	0	0	0
				8/10	0	0	0	8/3	0	0	0	8/9	0	0	0
				8/17	0	0	0	8/10	0	0	0	8/16	0	0	0
				8/24	0	0	0	8/19	0	0	0	8/23	0	0	0
				8/31	0	0	0	8/24	0	0	0	8/30	0	0	0
				9/9	0	0	0	8/31	0	0	0	9/5	0	0	0
				9/14	0	0	0	9/9	0	0	0	9/12	0	0	0
				9/21	0	0	0	9/14	0	0	0	9/20	0	0	0
				9/28	0	0	0	9/21	0	0	0	9/27	0	0	0
				10/5	0	0	0	9/28	0	0	0	10/4	0	0	0
				10/13	0	0	0	10/6	0	0	0	10/11	0	0	0
				10/19	0	0	0	10/13	0	0	0	10/18	0	0	0
				10/26	0	0	0	10/20	0	0	0	10/25	0	0	0
				11/2	0	0	0	10/26	0	0	0	11/1	0	0	0
				11/9	0	0	0	11/3	0	0.5	0	11/8	0.25	4	0
				11/16	0	0	6.66	11/10	1.75	2.75	0	11/16	0	1.25	0
				11/23	0	0.5	13.33	11/17	0	0	0	11/22	0	1.25	6.66
				11/30	0	0	26.66	11/23	0	0	0	11/29	0.25	0.5	6.66

Table 1. Contd.

	12/7	0	0	0	11/30	0	0	0	12/6	1	4.25	33.33
	12/14	0.25	0.25	33.33	12/7	0	0	6.66	12/13	2	3.25	26.66
	12/21	0	0.25	80	12/15	0.25	4.25	100	12/20	19.25	29.25	93.33
	12/23	0	0	100	12/21	2.5	12	100	12/28	3.25	1.75	100
	12/28	0	0.75	93.33	12/29	1.75	5.75	100				

Table 2. Mean adult insects (male and female) trapped per day (MAD) e percentage of eggs infestation (%) of *Drosophila suzukii* on the raspberry from Vacaria, RS, Brazil in 2014 to 2017.

2014				2015				2016				2017			
Data	**M**	**F**	**%**	**Data**	**M**	**F**	**%**	**Data**	**M**	**F**	**%**	**Data**	**M**	**F**	**%**
8/6	0	0	0	1/5	24.25	14	40	1/4	4.5	12.75	66.66	1/5	11	9	13.33
8/12	0	0	0	1/12	9.5	3.25	40	1/11	2	4	60	1/11	11.25	6.5	33.33
8/19	0.25	0	0	1/19	3.5	2.25	40	1/18	2.25	2.5	86.66	1/18	21.5	20.25	0
8/26	0	0	0	1/26	2.75	1.5	40	1/25	0.5	0.75	46.66	1/26	7.25	18	6.66
9/5	0	0	0	2/2	1.75	1	0	2/1	0.25	0.25	20	2/2	7.75	13.75	20
9/15	0	0	0	2/9	0.25	1.5	0	2/10	0	0.25	13.33	2/9	13	13.75	0
9/22	0	0	0	2/23	1.5	6.5	40	2/15	1	0	6.66	2/16	8.5	10	73.33
9/29	0.25	0	0	3/2	6.5	8	40	2/22	1.75	1	26.66	2/22	9.75	10.5	40
10/6	0.25	0	0	3/9	3	1.75	20	2/29	2	1.25	26.66	3/3	6	4.25	26.66
10/13	0	0	0	3/16	9.25	2	93.33	3/7	1.25	2.25	6.66	3/8	6	2.75	66.66
10/21	0	0	0	3/23	16.25	4.25	66.66	3/14	4	4.75	66.66	3/15	5	2.75	46.66
10/27	0	0	0	3/30	2.75	3	86.66	3/21	1.5	1.5	73.33	3/22	15.75	21.25	33.33
11/3	0	0.5	2	4/6	18	5.75	80	3/28	9.75	6.5	86.66	3/29	9.25	7.25	100
11/10	0	0	8	4/13	12.5	3.5	80	4/4	10	3.5	33.33	4/5	16.5	6.25	86.66
11/17	0.25	0	6	4/22	4.75	1.25	6.66	4/11	0	0.75	33.33	4/13	27	14.5	80
11/24	0.25	0.75	0	4/27	17.25	5.25	20	4/21	4	2.75	33.33	4/19	15.75	21	80
12/1	0	0	0	5/4	3.25	1.25	0	4/28	0	0	0	4/26	12	14.5	80
12/8	0.25	1.25	0	5/11	2.75	1.75	0	5/4	2.5	0.5	0	5/4	8.25	5.75	33.33
12/15	3.75	1.75	53.33	5/18	5.25	2	0	5/12	0	0.25	0	5/10	12.25	6	80
12/19	3.5	3.5	46.66	5/25	0.5	1.5	6.66	5/18	0	0	0	5/17	6.25	5	33.33
12/26	4	5.25	26.66	6/1	0	0.75	0	5/27	0.25	0	0	5/24	5.5	4.5	20
				6/8	0	0	0	6/2	0.25	0	0	5/31	11.5	7.25	20
				6/15	0	0.5	0	6/8	0	0	0	6/7	6.25	6.25	26.66
				6/22	0	0.25	0	6/16	0	0	0	6/14	0.25	0	0
				6/29	0	0	0	6/22	0	0	0	6/21	0	0	0
				7/6	0	0.5	0	6/29	0	0	0	7/5	0	0	0
				7/13	0	0	0	7/6	0	0	0	7/12	0	0	0
				7/21	0	0	0	7/13	0	0	0	7/18	0	0	0
				7/27	0	0	0	7/20	0	0	0	7/26	0	0	0
				8/3	0	0	0	7/27	0	0	0	8/2	0	0	0
				8/10	0	0	0	8/3	0	0	0	8/9	0	0	0
				8/17	0	0	0	8/10	0	0	0	8/16	0	0	0
				8/24	0	0	0	8/19	0	0	0	8/23	0	0	0
				8/31	0	0	0	8/24	0	0	0	8/30	0	0	0
				9/9	0	0	0	8/31	0	0	0	9/5	0	0	0
				9/14	0	0	0	9/9	0	0	0	9/12	0	0	0
				9/21	0	0	0	9/14	0	0	0	9/20	0	0	0
				9/28	0	0	0	9/21	0	0	0	9/27	0	0	0
				10/5	0	0	0	9/28	0	0	0	10/4	0	0	0

Table 2. Contd.

	10/13	0	0	0	10/6	0	0	0	10/11	0	0	0
	10/19	0	0	0	10/13	0	0	0	10/18	0	0	0
	10/26	0	0	0	10/20	0	0	0	10/25	0	0	0
	11/2	0	0	6.66	10/26	0	0	0	11/1	0	0	0
	11/9	0	0	6.66	11/3	0	0	0	11/8	5.25	12	0
	11/16	0	0	0	11/10	0	0	0	11/16	3.5	5.5	40
	11/23	0	0	0	11/17	0	0	0	11/22	17	16.75	0
	11/30	0.25	0.75	0	11/23	0	0	0	11/29	5.75	5.75	26.66
	12/7	0.25	0.75	33.33	11/30	0	0	0	12/6	14	10.25	40
	12/14	0.75	0	53.33	12/7	0.25	1.5	46.66	12/13	5	1.75	86.66
	12/21	1	1.5	66.66	12/15	3.75	7.25	26.66	12/20	24.5	22.75	66.66
	12/23	3	4	0	12/21	4	9.75	66.66	12/28	10.75	8.75	73.33
	12/28	3.75	5.75	0	12/29	6.75	10.25	20				

Table 3. Mean adult insects (male and female) trapped per day (MAD) e percentage of eggs infestation (%) of *Drosophila suzukii* on the strawberry from Vacaria, RS, Brazil in 2014 to 2017.

2014				2015				2016				2017			
Data	**M**	**F**	**%**	**Data**	**M**	**F**	**%**	**Data**	**M**	**F**	**%**	**Data**	**M**	**F**	**%**
8/6	0	0	0	1/5	1	1	0	1/4	0.75	1.25	46.66	1/5	1.25	0.875	13.33
8/12	0	0	0	1/12	0.5	0	0	1/11	0.25	0.5	86.66	1/11	1.625	1.375	0
8/19	0	0	0	1/19	0.75	0.25	0	1/18	1.5	3.5	60	1/18	1.625	1.625	0
8/26	0	0	0	1/26	0.5	0.75	0	1/25	0.25	0	53.33	1/26	1.75	1.125	100
9/5	0	0	0	2/2	0	0	0	2/1	0	0	13.33	2/2	0.625	1.125	33.33
9/15	0	0	0	2/9	0.25	0.5	0	2/10	0.25	0.25	6.66	2/9	1.125	2.375	13.33
9/22	0	0	0	2/23	0	1.75	33.33	2/15	0	1.75	0	2/16	0.75	0.25	46.66
9/29	0	0	0	3/2	0.25	0.25	13.33	2/22	0	0	46.66	2/22	0.875	2.375	26.66
10/6	0	0	0	3/9	0.25	0.25	0	2/29	0	0	53.33	3/3	0.875	3.5	46.66
10/13	0	0	0	3/16	4.25	3.5	66.66	3/7	0.25	0.25	20	3/8	0.75	2.625	40
10/21	0	0	0	3/23	1.5	2.5	0	3/14	0	0.25	60	3/15	1.5	2.5	40
10/27	0	0	0	3/30	0.75	0	53.33	3/21	0.25	0.25	26.66	3/22	2	2.375	40
11/3	0	0	0	4/6	1.75	0.5	86.66	3/28	0.25	0.5	26.66	3/29	10.125	3.25	86.66
11/10	0	0	0	4/13	0.25	0	20	4/4	0.75	0.5	20	4/5	4.125	3.75	40
11/17	0	0	0	4/22	0	0	53.33	4/11	0	0	13.33	4/13	9.875	5	60
11/24	0	0	0	4/27	0	0	66.66	4/21	0	0.75	66.66	4/19	2	3.375	66.66
12/1	0	0	0	5/4	0	0	13.33	4/28	0.125	0	0	4/26	1	0.625	73.33
12/8	1.25	0.5	0	5/11	0	0	40	5/4	0	0	0	5/4	2.75	1.5	6.66
12/15	0	0.25	13	5/18	0	0	0	5/12	0	0	0	5/10	1.75	1.5	13.33
12/19	0	0	0	5/25	0	0	0	5/18	0	0	0	5/17	0.625	0.5	26.66
12/26	0.75	1	0	6/1	0	0	0	5/27	0	0	0	5/24	0.5	0.25	6.66
				6/8	0	0	0	6/2	0	0	0	5/31	0.125	0.25	0
				6/15	0	0	0	6/8	0	0	0	6/7	0.375	0.5	0
				6/22	0	0	0	6/16	0	0	0	6/14	0.125	0	0
				6/29	0	0	0	6/22	0	0	0	6/21	0	0	0
				7/6	0	0	0	6/29	0	0	0	7/5	0	0	0
				7/13	0	0	0	7/6	0	0	0	7/12	0	0	0
				7/21	0	0	0	7/13	0	0	0	7/18	0	0	0
				7/27	0	0	0	7/20	0	0	0	7/26	0	0	0
				8/3	0	0	0	7/27	0	0	0	8/2	0	0	0

Table 3. Contd.

				8/10	0	0	0	8/3	0	0	0	8/9	0.125	0	0
				8/17	0	0	0	8/10	0	0	0	8/16	0	0	0
				8/24	0	0	0	8/19	0	0	0	8/23	0	0	0
				8/31	0	0	0	8/24	0	0	0	8/30	0	0	0
				9/9	0	0	0	8/31	0	0	0	9/5	0	0	0
				9/14	0	0	0	9/9	0	0	0	9/12	0.125	0	0
				9/21	0	0	0	9/14	0	0	0	9/20	0	0.75	0
				9/28	0	0	0	9/21	0	0	0	9/27	0.125	0.625	0
				10/5	0	0	0	9/28	0	0	0	10/4	0	0	6.66
				10/13	0	0	0	10/6	0	0	0	10/11	0.625	0.625	0
				10/19	0	0.25	0	10/13	0	0	0	10/18	0	0	0
				10/26	0	0	0	10/20	0	0	0	10/25	0	0	0
				11/2	0	0	0	10/26	0	0	0	11/1	0	0	0
				11/9	0	0	0	11/3	0	0	0	11/8	0.5	0	0
				11/16	0	0	0	11/10	0	0	0	11/16	0	0	0
				11/23	0	0	0	11/17	0	0	0	11/22	0	0	0
				11/30	0	0.25	20	11/23	0	0	0	11/29	0	0	0
				12/7	0	0.25	20	11/30	0	0	0	12/6	0	0	0
				12/14	0	0.5	26.66	12/7	0	0.125	33.33	12/13	0	0	0
				12/21	0	0	13.33	12/15	0.625	1.375	20	12/20	0	0	0
				12/23	0.25	0.75	46.66	12/21	0	0.5	6.66	12/28	0.125	0.25	0
				12/28	0	1.25	33.33	12/29	3.375	6.5	20				

Table 4. Mean adult insects (male and female) trapped per day (MAD) e percentage of eggs infestation (%) of *Drosophila suzukii* on the blueberry from Vacaria, RS, Brazil in 2014 to 2017.

2014				**2015**				**2016**				**2017**			
Data	**M**	**F**	**%**	**Data**	**M**	**F**	**%**	**Data**	**M**	**F**	**%**	**Data**	**M**	**F**	**%**
8/6	0	0	0	1/5	3	2.75	0	1/4	2	13.25	0	1/5	1.75	1.5	0
8/12	0	0	0	1/12	0	0	0	1/11	2.25	3.5	0	1/11	0	0	0
8/19	0	0	0	1/19	0	0	0	1/18	0.25	2.5	0	1/18	0	0	0
8/26	0	0	0	1/26	0	0	0	1/25	0	0	0	1/26	0	0	0
9/5	0	0.75	0	2/2	0	0	0	2/1	0	0	0	2/2	0	0	0
9/15	0	0	0	2/9	0	0	0	2/10	0.25	0.25	0	2/9	0	0	0
9/22	0.25	0	0	2/23	0	0	0	2/15	0	0	0	2/16	0	0	0
9/29	0	0	0	3/2	0	0	0	2/22	0	0.75	0	2/22	0	0	0
10/6	0	0	0	3/9	0	0	0	2/29	0	0.5	0	3/3	0	0	0
10/13	0	0	0	3/16	0	0	0	3/7	0	0.25	33.33	3/8	0	0	0
10/21	0	0.25	0	3/23	0	0	0	3/14	0	0.5	0	3/15	0	0	0
10/27	0	0	0	3/30	0	0	0	3/21	0.25	0.25	0	3/22	0	0	0
11/3	0	0.25	0	4/6	0	0	0	3/28	0	0.75	0	3/29	0	0	0
11/10	0	0	0	4/13	0	0	0	4/4	0.75	0.5	0	4/5	0	0	0
11/17	0	0	0	4/22	0	0	0	4/11	0	0	0	4/13	0	0	0
11/24	0	0	0	4/27	0	0	0	4/21	0.75	0.75	0	4/19	0	0	0
12/1	0	0	0	5/4	0	0	0	4/28	0	0	0	4/26	0	0	0
12/8	0	0.5	0	5/11	0	0	0	5/4	0	0	0	5/4	0	0	0
12/15	0	0.25	13.33	5/18	0	0	0	5/12	0	0	0	5/10	0	0	0
12/19	0	0	26.66	5/25	0	0	0	5/18	0	0	0	5/17	0	0	0
12/26	0.75	0.5	60	6/1	0	0	0	5/27	0	0	0	5/24	0	0	0
				6/8	0	0	0	6/2	0	0	0	5/31	0	0	0

Table 4. Contd.

	6/15	0	0	0	6/8	0	0	0	6/7	0	0	0
	6/22	0	0	0	6/16	0	0	0	6/14	0	0	0
	6/29	0	0	0	6/22	0	0	0	6/21	0	0	0
	7/6	0	0.5	0	6/29	0	0	0	7/5	0	0	0
	7/13	0	0	0	7/6	0	0	0	7/12	0	0	0
	7/21	0.25	0	0	7/13	0	0	0	7/18	0	0	0
	7/27	0	0	0	7/20	0	0	0	7/26	0	0	0
	8/3	0.25	0	0	7/27	0	0	0	8/2	0	0	0
	8/10	0	0	0	8/3	0	0	0	8/9	0	0	0
	8/17	0.25	0	0	8/10	0	0	0	8/16	0	0	0
	8/24	0	0	0	8/19	0	0	0	8/23	0	0	0
	8/31	0.25	0	0	8/24	0	0	0	8/30	0	0	0
	9/9	0	0	0	8/31	0	0	0	9/5	0	0	0
	9/14	0.25	0	0	9/9	0	0	0	9/12	0	0	0
	9/21	0	0.25	0	9/14	0	0	0	9/20	0	0	0
	9/28	0	0.5	0	9/21	0	0	0	9/27	0	0	0
	10/5	0	0	0	9/28	0	0	0	10/4	0	0	0
	10/13	0	0	0	10/6	0	0	0	10/11	0	0	0
	10/19	0	0	0	10/13	0	0	0	10/18	0	0.25	0
	10/26	0	0	0	10/20	0	0	0	10/25	0	0	0
	11/2	0	0	0	10/26	0	0	0	11/1	0	0	0
	11/9	0.25	0	0	11/3	0	0.25	0	11/8	0	0	0
	11/16	0	0	0	11/10	0.25	0.5	0	11/16	0	0	0
	11/23	0	0.5	0	11/17	0.25	3.75	0	11/22	0	0	0
	11/30	0	0	0	11/23	0	0	0	11/29	0	0	0
	12/7	0	0.25	33.33	11/30	0	0.5	0	12/6	1.25	1.25	0
	12/14	0.25	0.5	0	12/7	0	1.75	6.66	12/13	0	0	0
	12/21	0.25	1	0	12/15	0	0	0	12/20	0	0	0
	12/23	0	1.5	0	12/21	0	2.5	0	12/28	0	0	0
	12/28	7	15.75	0	12/29	0	0.75	0				

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Full Length Research Paper

Maize ideotype breeding for changing environmental conditions

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The demand for corn in the developing world will double by the end of 2050. The challenges due to the climate change are real. Where extreme weather events will become more frequent and climate change projections suggest that large yield losses occur in many regions of the world. Corn is cultivated throughout the world and is a strategic crop: can tolerate high radiation intensities and exhibits high efficiency in the use of water. A framework is needed to design maize ideotypes for site specific condition with the definitions of past, present and future environmental history, and the response of the local material using empirical or mechanistic modeling. The ideotype is a combination of different types of biological traits or the genetic basis that confer enhanced performance for a particular biophysical environment, specific cropping system and end use of the crop. Studies of genotype performance under climate variability always shows that a single trait will never improve plant performance in all climatic scenarios and similarly a single genotype will not cope with all the existing climatic variability. In the past, ideotyping was based on visual and growth phenotypes, but future ideotyping trend will focused more on the knowledge of the genotype. In the future, the strategies for ideotipificación will be based on strong biotechnological techniques facilitated by the bioinformatics, filling gaps in the current knowledge and overcoming the climatic change challenges and increased the world population.

Key words: Maize, ideotype, climate change.

INTRODUCTION

Donald (1968) first time coined the term ideotype for the first time while Mark and Pearce (1975) proposed ideal plant type of maize. The later proposed ideal maize plant with small tassel size, low tillers, large cobs and angled leaves for good light interception. A maize ideotype that can utilize an optimum production environment should be a package of:

(1) Stiff, vertically-oriented leaves above the ear (leaves below the ear should be horizontally oriented);
(2) Maximum photosynthetic efficiency;
(3) Efficient conversion of photosynthate to grain;
(4) Short interval between pollen shed and silk emergence;
(5) Ear-shoot prolificacy;
(6) Small tassel size;
(7) Photoperiod insensitivity;
(8) Cold-tolerance in germinating seeds and young

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seedlings (for genotypes grown in areas where early-planting would require planting in cold, wet soils);
(9) As long as a grain-filling period is practically possible and;
(10) Slow leaf senescence (Mark and Pearce, 1975).

This environment should include optimum conditions of moisture, temperature, fertility etc. As ideal conditions do not always prevail, the aim is to ideotype for unpredictable stressed environment to escape any threat of food security in future. To identify maize ideotypes, a multi-disciplinary approach is essential (Cairns et al., 2012). Many scientists have made first hypothetical approaches to reach the idea of ideotype (Jonathan, 2012), and then identified the required source fitting the frame. A mathematical model of plant growth could be possibly used to design ideotypes and thus lead to new breeding strategies based on the guidance from optimization techniques. Certain optimization approaches relying on plant growth models, green lab model, source sink dynamics etc, may help improve breeding strategies and design ideotypes of high-yield maize (Rui et al., 2010). In crop modelling under climate change, quantitative trait loci (QTLs) can identify the best ideotypes as we need 5 to 15 years to breed a variety, and 2050 is only at two to eight cycles of breeding (Chapman, 2011).

Effect of climate change on the world maize production

According to a report (Annynomus, 2014), until 2050, extreme weather events will become more frequent and extreme. They will include mega-droughts, deadly heat waves and a year's rainfall in a month in some places. If greenhouse gas emissions continue to increase at the current rate, the average global temperatures could have risen more than 4°C. Climate change impacts will be worst in countries already suffering high levels of hunger. Extreme weather events are likely to become more frequent in the future and will increase risks and uncertainties within the global food system (Tim and Joachim, 2013).

Statistical studies of rainfed maize yields in the world and particularly in United States have an indication of strong negative yield response to temperatures above 30°C (David et al., 2013). Climate change has a universally negative effect on agriculture. In china, reduction of corn production in the Northeast region due to this negative effect is predicted (Xiang et al., 2014).

The average maize yield in the west and central regions in China is projected to decrease to 15% or more by 2050 as predicted by 90% of 120 projected scenarios. In the long run, the maize cultivars need to be introduced in line with the future warming climate (Meng et al., 2013). It requires advance regional policies and strategies until 2030 to mitigate this possible predicted reduction (Xiang et al., 2014).

Iowa's main corn growing state in the USA region represents the ideal climate and soils for corn production that contributes substantially to the world corn economy. The prediction is a decline in maize yields from the late 20th century to the mid and late 21st century, ranging from 15 to 50%. To maintain crop yields, farmers will need a set of adaptation strategies (Hong et al., 2016).

Climate change projections suggest that large yield losses will occur in many regions, particularly within sub-Saharan Africa and South Asia (Cairns et al., 2012). Agriculture "the pillar of economy" is also under threat in Malawi. This region of Africa is supposed to face 33% of losses in corn production due to 14% less rain and climate change at the end of the century. There is need to plan supplementary irrigation strategies, crop diversification and natural conservation methods (Kondwani et al., 2016).

The climate change is also of great concern in the Kingdom of Swaziland. The rainfall variability is a threat to their staple food "corn". The 60% rainfall is recorded in just two months of a year. This erratic rainfall results in a decrease in corn production. To mitigate climate change and increase family food security need is of soil conservation, intercropping, cultivation of short-season maize varieties / early maturation, diversification of crops such as millet and sorghum, etc. (Oseni and Masarirambi, 2011). Average temperature will negatively affect the corn crop in Pakistan, producing a 6% reduction in corn production until the year 2030. This scenario requires the key political intervention of the government to address climate change in agriculture and particularly in corn (Shakoor et al., 2017).

Climate change is the most serious environmental threat globally. The increasing global population with increase in earth mean temperatures (between 1.8 to 4.0°C) is a burning issue. Despite the technological success in the previous half of the 20th century, the agricultural production and economy is still highly susceptible to the predicted climate change (Rebolledo, 2014). Studies of genotype performance under climate variability always shows a single trait will never improve plant performance in all climatic scenarios and similarly a single genotype will not cope with all the existing climatic variability.

Root system

A maize root system efficient enough to absorb and store much water and N during hours of its availability before wasting into deeper soil strata should be breeder's aim. Ideotype root architecture for efficient N and water acquisition in maize should include:

(1) Deeper roots with high activity that are able to uptake

NO_3^{-1} nitrate and water before it moves downward into deep soil;

(2) Vigorous lateral root growth under high water and N input conditions so as to increase spatial N and water availability in the soil;

(3) Strong response of lateral root growth to localized N and water supply so as to utilize their uneven distribution especially under limited conditions;

(4) Being able to establish symbiotic relationships with soil micro- organisms (MI et al., 2010).

Moreover, it should compete with weeds. Exploit genetic variability for competitiveness and early coleoptilar node with extensive shoot born root (Hochholdinger et al., 2004). Lodging can be a major factor affecting grain yield. Vigorous root system with ideal root anchor is required to overcome root lodging under adverse environment. Rtcs (root hair less), Bk2 and Rth3 (mineral uptake) are the genes identifying the target for ideotyping (Hochholdinger and Tuberosa, 2009).

N uptake efficiency

N, a limiting and essential nutrient to plant growth leads to annual application of an estimated 10 million metric tons of N fertilizer to the maize crop worldwide (Anonymous, 2012, 2014). Although a much smaller amount of N fertilizer is absorbed by roots and very little amount is utilized by plant as compared to the N applied. Instead of increasing N application we should focus on an ideotype with very active N uptake efficiency. The extensive use of N fertilizer increases crop input costs and also have negative impact on soil, water and air quality at both local and ecosystem scales (Tilman et al., 2002). The manufacture of N fertilizer with natural gas is an energy-intensive process that is becoming increasingly costly. Reducing the amount of supplemental N used in maize production will have significant positive economic and environmental benefits to world agriculture. Indicating that more than half of the fertilizer N applied in maize crop production is lost to the environment. Thus, there is considerable opportunity for enhancing maize nitrogen use efficiency (NUE). The development of “N-fixing maize” is not far away as biotechnology has and will continue to play an important role in improving NUE (Alan and Brian, 2010).

Heat stress tolerance

Tolerance mechanisms help to fight plant tissues against dehydration. This type of tissue “hardening” occurs through the accumulation of proteins such as the dehydrins (hydrophilins) and heat shock proteins, and a wide range of compatible solutes (for example, polyols, glycine betaine, proline, inositol). By increasing the level and activity of enzymes and pathways, the plant can protect its tissues from the generation of potentially damaging reactive oxygen species (ROS) that are generated during periods of water limitation and stomatal closure (that is, ROS protective systems, GABA shunt, photorespiration).

Crassulacean acid metabolism (CAM) photosynthesis, variation of stomatal distribution and conductance, leaf cuticle properties (wax, hairs, boundary layers), hydraulic conductivity, leaf architecture (thickness, size, area, rate of appearance, leaf rolling, erectness), and canopy architecture are the set of traits that can modulate water utilization (Jordan et al., 1983). Maize crop is most sensitive to environmental stresses. Maize plants tend to experience extreme sensitivity to water deficit, during a very short critical period, from flowering to the beginning of the grain-filling phase. Maize crops tend to have the highest water requirement during the critical period, when the maximum leaf area index combines with the highest evaporative demand.

Drought avoidance traits have a significant impact on yield, because they help plants maintain good water status, allowing continued photosynthesis, growth, and development. Dehydration tolerance is important during seedling establishment for improved stand and maximum germination.

Traits that help plants avoid water deficit, such as the establishment of a deep rooting system have a greater impact on yield, assuming in the case of deep roots that water is available in the soil profile and soil water content is recharged annually (Sinclair and Muchow, 2001), (Sharp, 2002).

‘Staygreen’ trait in Sorghum has a vital impact on yield in water- limited environments because this response improves plant water status, photosynthetic activity, and N uptake in water-limited environments during the reproductive phase (Alan and Brian, 2010). Genes/traits contributing high grain yield and drought stress conditions can be identified in several ways for example, mutants with modified response to water limitation in field, QTL mapping and inserting the cloned genes into the desired germplasm, screening by comparative analysis and artificial stress.

Genes that show modified expression in response to water deficit are the easiest to identify. However, determining the importance of a specific inducible gene with regard to yield in water-limiting environment is challenging. One of these pathways starts with perception of water deficit through reduction of cell turgor, and this leads to accumulation of the plant hormone abscisic acid (ABA).

ABA in turn activates a signaling pathway that reduces stomatal aperture, contributes to differential root/shoot growth (Sharp, 2002) and modulates gene expression. Expression of putative stress tolerance genes using promoters that are activated in response to water deficit (or ABA) has been more successful in enhancing tolerance without secondary effects.

Sorghum closes its stomata in water deficient response but become nearly insensitive following anthesis. This change in sensitivity allows continued CO_2 fixation and grain filling even under drought stress. This can otherwise results in stomatal closer, inhibit photosynthesis, reduce sugar levels, and cause complete loss of reproductive structures. After identification of these traits and genes their optimization is essential in terms of tissue/cell- specific expression and expression during plant development (Alan and Brian, 2010).

Plant height

Crop yield potential may be increased by reducing plant height and selecting for erect leaves. Dwarf genes provided by the elite maize inbred line Shen5003 have been successfully exploited to develop several inbreeds and hybrids with reduced plant height. Recent genetic analysis and molecular characterization of dwarf mutants in maize revealed that mutations in dwarf genes including d1 (gibberellin-responding dwarf gene), d2, d3, d5, d8 (GA-insensitive dwarf genes), d9, An1, DWF1 and DWF4 (Jianfeng et al., 2011). Dwarf maize mutants are short, compact plants with shortened internodes, short wide leaves, and short erect tassels. Plant height, cob height and tassel size are important characters contributing towards stem lodging. Semi-dwarf with low bearing cobs and light tassels are attributes supporting lodging resistance (Janick, 2004).

Tassel and cob architecture

To ensure high quality F1 seed production, the ideal male parent should have a relatively large tassel with excellent amount of pollen viable for a longer period of time. The ideal female parent should be with large ear, producing a large number of kernels and relatively small tassel to direct more energy toward grain yield.

Ideotyping for grain yield should target smaller tassels, as tassel size, tassel weight, and tassel branch number are negatively associated with it. It is important that pollinators should have a very large tassel with excellent quality and quantity of pollens. Tassel branch length and spikelet pair density along with variation in branch angle are important as they determines the area, the pollen can be dispersed and also plays a role in shading of the flag leaf. This type of variation is associated with ra2 (Upadyayula et al., 2005).

The components of ear inflorescence architecture such as kernel row number, number of kernels per row, and kernel number density are positively correlated with grain yield. Previous quantitative genetic studies suggested indirect election for greater yield that involved selections of some ear traits could be more effective than direct selection for yield itself, because of lower heritability of yield. A long-term divergent selection for ear length indicated that grain yield did not increase with selection for longer ear length, but yield decreased significantly with selection for shorter ear length (Lopez-Reynoso and Hallauer, 1998).

Summarizing 20 cycles of divergent mass selection for seed size, reported that grain yield did not increase with selection for greater seed size, but grain yield decreased significantly with selection for small seed size. Significant positive correlations between grain yield and kernels per row and kernel rows per Ear is present. Fasciated Ear2 (FEA2) is responsible for more kernel rows per cob (18 to 20). Insertion of this gene through cloning into a wild type with 256 kernels yield increases of 13% with cob bearing 289 kernels (Bommert et al., 2013).

Prolific hybrids out-yield non-prolific types and are more drought stress tolerant. Larger grain weight per plant is due to more kernels per plant in the reduced-input system, and a combined effect of more kernels and heavier 1000-kernel weight per plant in the high-input system. Improved kernel number per plant for prolific hybrids was associated with kernels from secondary ears. Grassy tiller1 (gt1) suppresses the initiation of multiple ear per plant and only 1 or 2 ear will develop (Janick, 2004).

Quality traits

Majority of the seed phosphates are present in the form of phytic acid, digested only by rudiatary animals but remain undigested in poultry and human feed. Inorganic P is digest-able and is controlled by *lpa* alleles (Raboy, 2006). Similarly, quality protein maize (QPM) should be the priority. It contains nearly twice as much usable protein as other maize and yields 10% more grain than traditional varieties of maize. The deficient protein quality due to low lysine and tryptophan in maize grain can be improved by replacing normal Opaque2 (O2) alleles with nonfunctional mutant O_2 alleles (Prasanna et al., 2001). Similarly, waxy maize with recessive *wx* allele can increase maize industrial demand as a byproduct.

Transgenic corn

The first transgenic corn hybrid with insect resistance traits was commercialized in 1996 in the USA (Mendelsohn et al., 2003), (James, 2006). These products were targeted at lepidopteran pests of corn, particularly stem borers that are difficult to control using conventional insecticides. Now transgenic Bt corn hybrids have been adopted on tens of millions of hectares (James, 2006).

In addition, Bt corn hybrids containing coleopteran-active insecticidal proteins that control the larvae of the damaging corn rootworm complex (*Diabrotica* spp.) have been developed. Increasingly, corn farmers are purchasing hybrids with combinations of these insect resistance traits (both lepidopteran and coleopteran pest protection), along with herbicide-tolerance traits for improved weed control (The event MON 88017 also expresses Cry3Bb1 but combines a Roundup herbicide tolerance gene in the same expression cassette) (Brookes and Barfoot, 2007; James, 2006).

Even though these products have an obvious technical fit in many countries, the regulatory systems are not always in place to approve such products, and distributional and educational challenges exist when it comes to getting the products in farmers' hands (particularly in Africa and Asia). Ideotype may be benefited with transgenic technology but novel partnerships will be needed, along with broad governmental involvement and assistance from international organizations (Alan and Brian, 2010).

Trait inheritance pattern

The global gene pool is the base material for selection, especially for plant stature, maturity durations, leaf size and angle. For the incorporation of desirable traits, knowledge of the association between various characters is essential before starting hybridization program. Inheritance pattern in most of the agronomic important traits shows non-additive gene effects. Over-dominance gene effects for days from silking to physiological maturity, days from anthesis to physiological maturity, plant height, kernel depth, number of rows per ear and grain yield. The most appropriate strategy for the exploitation of these effects is to obtain hybrid cultivars, and to evaluate these characteristics in hybrid combinations. The gene effect for days from emergence to physiological maturity and number of kernels per row is complete dominance, suggesting that reciprocal recurrent selection will be effective. Ear leaf area and ear length are controlled by partial dominance, indicating that additive gene effects are more important than non-additive gene effects for controlling the inheritance of these traits. Therefore, improvement of these traits through selection of breeding materials is highly feasible. Broad-sense heritability ranged between 47.4 and 89.4% for days to physiological maturity and number of rows per ear; however, narrow-sense heritability varied between 7.3 and 50.6% for days from anthesis to physiological maturity and ear leaf area, respectively (Zare et al., 2011).

Conclusion

One who knows the success story of QPM and transgenic maize will always believe on heat and drought stress tolerant maize, growing in adverse conditions, ensuring food security of millions. All breeder's dreams and plans that target future threats will evolve a new ideotype. This will carry the genetic background of all favorable alleles along with additional developments that breeder will contribute time by time. History of ideotyping is the history of breeder's success while future of breeder and food security is ideotyping.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Precocious screening tests of the resistance of two varieties of cocoa seedlings (*Theobroma cacao* L.) from combinations of fertilizers against *Phytophthora megakarya* (Brasier and Griffin) in nursery

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Experiments were conducted to appreciate the resistance level of cocoa seedlings aged three months from combinations of fertilizers against *Phytophthora megakarya* based on artificial leaf disk inoculation test. The two cocoa varieties involved were: Tafo 79/501 (V_1) and SNK 13 (V_2). For each variety, 11 treatments with three replicates were used on 33 randomly-selected plants. These include Arbuscular Mycorrhizal Fungi (M), *Trichoderma asperellum* strain PR11 (T), Organic Fertilizer Gro-wild (O), Chemical fertilizers (NPK), the following combinations (TO), (MT), (MO), (MOT), (MOTNPK) as well as a negative control made by original substrate only (T-) and sterile control with sterilized substrate (TS). Results show that the resistance level of cocoa seedlings in nursery to *P. megakarya* varied with the cocoa variety and for a given variety with the fertilizer. Fertilizers were classified into four categories according to the variation of the infection severity index from 0 (V_1MOT) to 4.72 (V_1TS) and from 1.27 (V_2MOT) to 4.86 (V_2T-). The most efficient treatment for both V_1 and V_2 was MOT that allowed a dualistic action between Arbuscular Mycorrhizal Fungi and *T. asperellum* in the presence of organic fertilizer Gro-Wild.

Key words: Cocoa, *Phytophthora megakarya*, leaf disk, infection severity index, resistance.

INTRODUCTION

Theobroma cacao L. is one of the most important cash crops in Cameroon, and other countries in Central and West Africa (Assoumou, 1997). Africa is the main producer of this product worldwide (Lass, 2004). Indeed, about 73% of world production comes from the Ivory Coast, Ghana, Nigeria and Cameroon (ICCO, 2011).

Since 1990, Cameroon has suffered a drastic drop in its cocoa production (Mossu, 1990) whose main causes, among others, are the rural exodus, the stopping of state subsidies to cocoa farmers, the falling price of cocoa kilogram, the aging of cocoa plantations, pests and various diseases (Tchameni et al., 2012).

Fungal diseases are one of the most important limiting factor of cocoa production (Kouakou et al., 2011). *Phytophthora megakarya* is one of the Oomycete pathogens reported on *Theobroma cacao* (Tchameni et al., 2012). It is the most virulent of *Phytophthora* species that was first reported as the causal agent of black pod disease in 1979 (Akrofi, 2015).

P. megakarya is only endemic to West and Central Africa (Tondje et al., 2007). Nyassé et al. (1999) used isozyme and random amplified polymorphic DNA (RAPD) markers to estimate the genetic diversity and structure among *Phytophthora* isolates from Ghana, Togo, Nigeria, Cameroon, Gabon and Sao Tomé. This pathogen has become the main yield-limiting factor in cocoa production in the sub region (Akrofi, 2015) with yield losses of 50 to 80% in Cameroon (Ndoumbé-Nkeng et al., 2004).

Thus, the menace of *P. megakarya* on cocoa is of great concern to cocoa farmers and scientists. *Phytophthora* spp. control is a major challenge for world cocoa cultivation, and selection of resistant material is a priority research theme for many producing countries (Nyassé et al., 1995). Indeed, yield losses of this crop and cost of controlling black pod disease affecting cocoa constitute a significant financial burden on agricultural enterprises and has serious socio-economic and environmental consequences wherever these pathogens are found (Akrofi, 2015).

Besides, many genetic and environmental factors on growth and quality of seedling were reported in different plant species (Bilir et al., 2004; Yazici, 2010; Dilaver et al., 2015; Tebes et al., 2015; Cercioglu and Bilir, 2016; Yilmazer and Bilir, 2016; Cetinkaya and Cercioglu, 2017).

The challenge for the next 50 years is to double agricultural production by respecting the rules of sustainable agriculture with low inputs and without risk to human health (Tilman et al., 2002). Thus, there is increased need for fundamental knowledge in order to develop effective and sustainable methods for the control of crop diseases. One possible strategy is to develop agricultural practices based on ecological processes such as the relationship between flowers and pollinators (Klein et al., 2007; Azo'o et al., 2011, 2012, 2017), the use of natural soil resources, plant nutrient recycling, plant material selection, and management of organic and inorganic inputs (Duponnois et al., 2012). Overall, the general policy for sustainable agricultural and rural development consists in diversifying production systems to make the best possible use of available local resources (De Silguy, 1997).

The main objective of this study is to find the protection methods of cocoa seedlings in the nursery by biological fertilization process. More specifically, the study leads us to:

1. Evaluate *in vitro*, the resistance level of cocoa seedlings against *P. megakarya* by testing leaf disks of plants from various combinations of fertilizers and
2. Compare the level of susceptibility between two studied cocoa varieties from these combinations.

MATERIALS AND METHODS

Study site

The study was conducted in a nursery of the experimental station of the Institute of Agricultural Research for Development (IRAD) of Nkolbisson (11°36' East and 3°44' North) during the small rainy season. Nkolbisson is a suburb which belongs to the Yaoundé 7th Sub-Division, the Mfoundi Division and the Centre Region of Cameroon.

The Centre Region of Cameroon has Yaoundé as its chief town; this city is also the political capital of Cameroon and the capital of the Mfoundi Division. The Centre Region extends between latitudes 3°47' to 3°36' north and between longitudes 11°10' to 11°45' east. The average altitude is 760 m (Létouzey, 1968).

The Centre Region belongs to the forest zone of Cameroun. The climate here is Guinean type including four seasons with two differently dry and rainy seasons: the brief rainy season (March to June) is followed by the short dry season (July to August) and the longer rainy season (September to November) is followed by the more extended dry season (November to March). The mean annual rainfall is about 2000 mm and the mean annual temperature 26.6°C. These climatic conditions are favorable for cocoa cultivation in the region.

Plant material

Two varieties of cocoa namely Tafo 79/501 and SNK 13 collected from the Centre SODECAO (Cocoa Development Company) of Mengang (Centre Region, Cameroon) were used. The first variety is resulting from the hybridization between Nanay 32 and Parinai 7 varieties, and the second one from Trinitario and Forastero (Blaha and Lotodé, 1976). Both cocoa varieties are among the most disseminated by the Centre SODECAO of Mengang.

Biological, organic and chemical materials

Biological, organic and chemical materials used are reported in Table 1.

Combinations of different treatments

The different combinations of treatments applied per pot for each

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Table 1. List of biological, organic and chemical materials.

Biological material		
Designation	**Function**	**Origin**
Gigaspora margarita	Biofertilizer	
Acaulospora tuberculata	Biofertilizer	Laboratory of Biological Control and Applied Microbiology
Trichoderma asperellum	Biofertilizer	IRAD, Nkolbisson
Phytophthora megakarya	Pathogen	
Organic and chemical materials		
Gro-wild	Organic fertilizer	
Ridomil	Fungicide	Shop of phytopharmaceutical products, Yaoundé
NPK	Chemical fertilizer	

variety were as follows:

1. Arbuscular Mycorrhizal Fungi or AMF (M) of two strains (*Gigaspora margarita* and *Acaulospora tuberculata*)
2. *Trichoderma asperellum* strain PR11 (T)
3. Organic Fertilizer made from fish namely Gro-wild (O)
4. Chemical fertilizers or positive control (NPK or T_+)
5. *T. asperellum* strain PR11 + Organic fertilizer (TO)
6. AMF + PR11 (MT)
7. AMF + Organic fertilizer (MO)
8. AMF + Organic fertilizer + PR11 (MOT)
9. AMF + PR11 + Organic fertilizer + Chemical fertilizer (MOTNPK))
10. Negative control: original substrate without any treatment (T-)
11. Sterile control: sterilized substrate without any treatment (TS)

Preparation of pots

The soil used for experimentation was brought from the forest undergrowth of Messassi, a neighborhood of Yaoundé. This substrate is the same as that supplied to Nkolbisson nurserymen. The pots were made from polythene bags 20 x 17 x 65 mm. Three randomized blocks of 11 treatments with three replicates each were used per variety.

A total of 66 pots were constituted, including 33 per variety. The seeds of V_1 and V_2 were pre-germinated for 18 days before being transplanted one per pot. 10 g of an inoculum containing AMF spores (*Gigaspora margarita* and *Acaulospora tuberculata*) were brought into 30 pots when transplanting of the pre-germinated plants.

Conidia of *T. asperellum* (PR11) harvested from the seven-day old cultures were introduced into beakers containing 200 ml of distilled water. After mechanical stirring for 1 minute, 5 ml of conidia solution of 5.10^{-7}mol/ml concentration were scraped into 11 Petri dishes and added into 30 pots provided for this treatment using a micropipette (Tchameni et al., 2011).

A mixture of the organic fertilizer (Gro-wild) in 15 L of water was carried out; 80 ml of this solution was added in each of the 30 pots provided for this purpose. The chemical fertilizer NPK (20-10-10), regarded as a positive control (T_+), has been spread into 12 pots at a dose of 10 g/pot, with 2 g per pot weekly. For the sterile substrate (TS), 6 pots were concerned; in these pots, the soil was sterilized by autoclaving at 121°C for 1 h with 2 replications in an interval of 24 hours. Finally, 6 pots were set up as the negative control (T-); here, the pots received no treatment.

The nursery was housed inside a greenhouse of the Regional Biocontrol and Apply Microbiology laboratory of IRAD-Nkolbisson (Yaoundé, Cameroon). Watering was done twice a week, with one-half litre of water measured per pot at the dawn and at the twilight.

In vitro evaluation of leaf disks test

The leaf test is an artificial inoculation method to assess the resistance of genotypes against infections (Nyassé et al., 1995). It was here to search for the incidence and severity of the disease on the leaves of cocoa plants in petri dishes following the protocol of Nyassé et al. (1995).

Three months old leaves were collected early in the morning before sunrise for both varieties. Leaf disks of 15 mm in diameter were laid in petri dishes. It was subsequently inoculated by depositing one drop of 10 µl of the zoospore suspension in the middle of each one. The device consisted of 11 treatments for varieties Tafo 79/501 and SNK 13 respectively, with 3 replicates per treatment.

Overall, 33 petri dishes each containing 12 leaf disks were constituted per variety as function of the treatment received by the seedling. Only, leaf disks issued from the positive controls (T+) received each an additional drop of 10 µl of Ridomil (R). To monitor and describe disease expression, observations were made 6 days after incubation at ambient temperature according to the recommendations of Tchameni et al. (2011). The rating scale from 0 to 5 developed by Nyassé et al. (1995) was used for evaluation:

1. No symptom development was rated 0
2. Penetration point observed at the inoculated site was rated 1
3. Network of points was rated 2
4. Weblike patch was rate 3
5. Mottle patch was rated 4
6. True patch (necrosis) was rated 5.

The disease severity was determined for each treatment by calculating the ratio of the sum of individual scores over the total number of leave disks used (Tchameni et al., 2012). The infection severity index was used to express the resistance level as follows: 1) highly resistant: 0 < index ≤ 1; 2) resistant: 1 < index ≤ 2; 3) moderately resistant: 2 < index ≤ 2.5; 4) susceptible: 2.5 < index ≤ 3.5; 5) highly susceptible: 3.5 < index ≤ 5 (Paulin et al., 2008).

Statistical analysis

Data collected was keyed into an Excel sheet and analysed using Statistica 6.0 software. The analysis of variance (ANOVA) was used for multiple comparisons of means; when the overall difference between the means was found significant, the analysis was

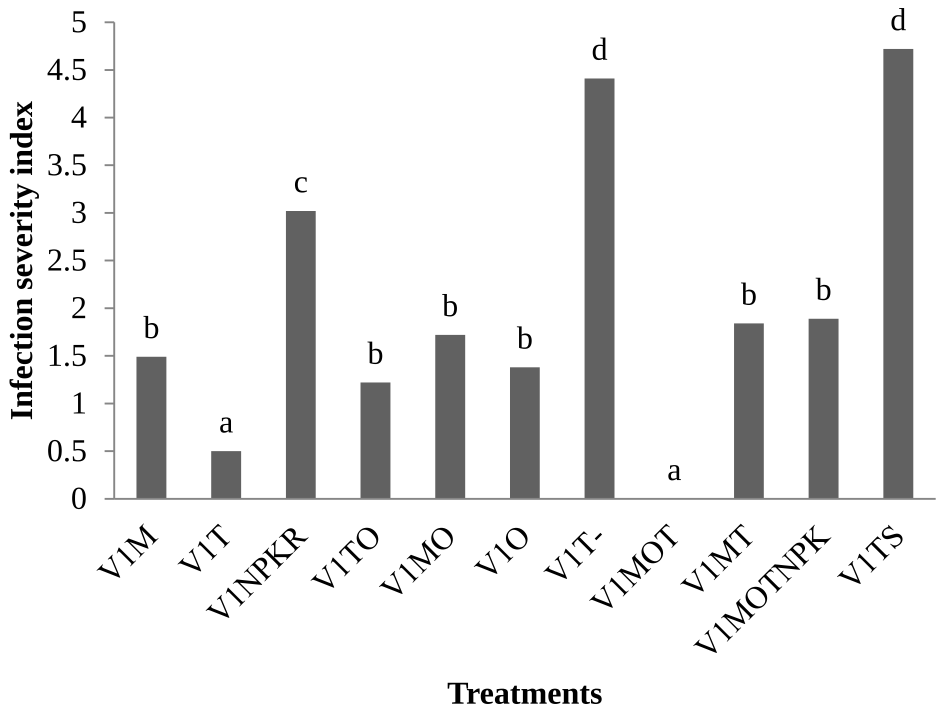

Figure 1. Infection severity index as a function of the treatments in Tafo 79/501 (Values followed with the same letter are not significantly different at *P* > 0.05, by HSD test).

Table 2. Categorization of treatments depending on the degree of susceptibility of V_1 to *P. megakarya.*

Treatments	Infection severity index	Degree of susceptibility
V_1MOT	0	Highly resistant
V_1T	0.5	
V_1TO	1.22	Resistant
V_1O	1.38	
V_1M	1.49	
V_1MO	1.72	
V_1MT	1.84	
V_1MOTNPK	1.89	
V_1NPKR	3.02	Susceptible
V_1T-	4.41	Highly susceptible
V_1TS	4.72	

continued by comparing the average pairwise using the HSD test. Means are reported as significantly different if *P* was less than or equal to 0.05.

RESULTS

From this study results, susceptibility of seedlings of cocoa of three months ageing in nursery varied with the different treatments applied. Figure 1 shows the variation of the infection severity index due to *P. megakarya in vitro*, depending on treatments in Tafo 79/501 (V_1) variety.

Analysis of variance shows that in Tafo 79/501, the manifestation of the disease is significantly different between treatments (F = 7.66, P < 0.05). Treatments are thus classified into four categories depending on the variation of the infection severity index of *P. megakarya* which determined the degree of susceptibility of different plants to the black pod disease. Smaller the index, largest

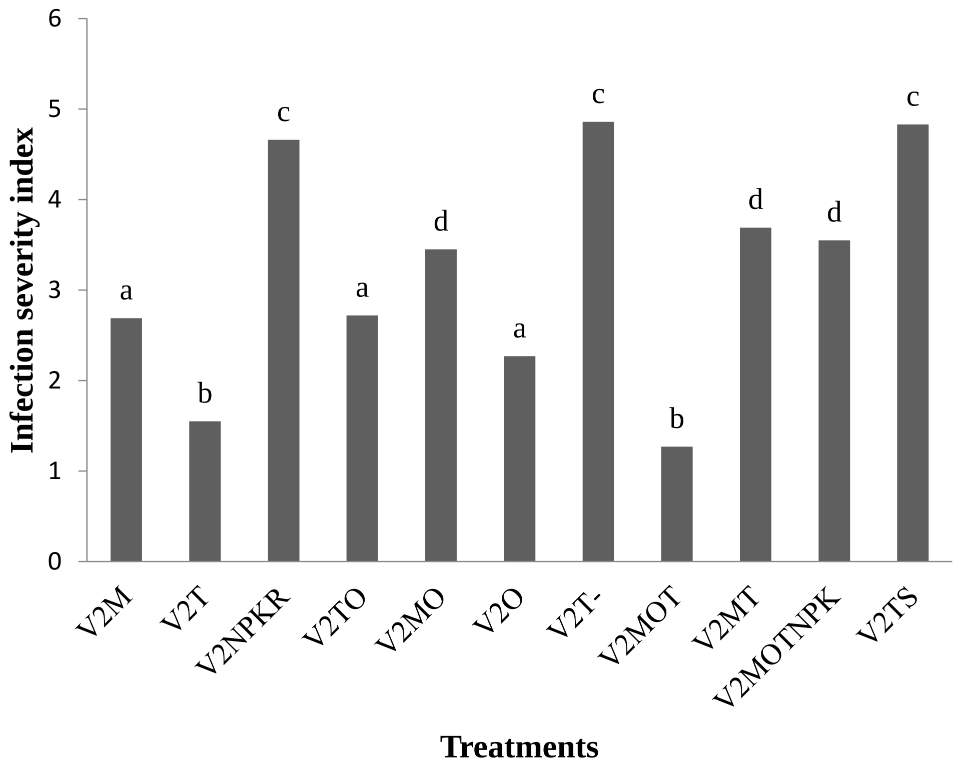

Figure 2. Infection severity index as a function of the treatments in SNK 13 variety (values followed with the same letter are not significantly different at *P* > 0.05, by HSD test).

the faculty of resistance acquired by the corresponding seedling plants; conversely, high index values determine the high susceptibility of plants to black pod disease (Table 2).

The treatment with *T. asperellum* (V_1T) and the mixture *T. asperellum*-Mycorrhizae-Organic fertilizer (Gro-wild) (V_1MOT) are those offering cocoa seedlings in the nursery high resistance to leaf development of *P. megakarya*. As it concerns treatment V_1MOT, no symptoms (0) were observed on correspondent leaf disks six days after inoculation of zoospores; about V_1T, beyond healthy leaf disks, some showed penetration points (1) of the germ *P. megakarya*.

Other treatments with Mycorrhizae and/or PR11 strain of *T. asperellum* following V_1M, V_1MO, V_1MOT, V_1MT, $V_1MTONPK$ and V_1TO as well as those based on organic fertilizer only (V_1O) make seedlings corresponding resistant to the development of *P. megakarya*. For those treatments, the leaf disks showed symptoms ranging from penetration points (1) to network of points (2).

The contribution of a drop of Ridomil on leaf disks of seedlings of cocoa which received chemical fertilizer NPK (V_1NPKR) gives unsatisfactory results. Here we observed the disks with network points (2), weblike patch (3) and mottle patch (4).

Finally, the substrate receiving no treatment (V_1T-) and the sterilized one (V_1TS) offer no protection to young corresponding plants and thus predispose them to a greater susceptibility to *P. megakarya*. This is why the bulk of the corresponding leaf disks showed true patch or necrosis (5) overall. Figure 2 shows the variation of the infection severity index due to *P. megakarya in vitro*, depending on treatments in SNK 13 (V_2) variety.

As regards of this variety, the differences were also significant between treatments (F = 6.43; P < 0.05). The results reported in Table 3 show the categories of each treatment. The treatment V_2T and V_2MOT offer cocoa seedlings resistance to the development of the germ of black pod disease, despite the presence of penetration points (1) and connected points (2) observed on leaf disks.

The treatment based on Organic fertilizer Gro-wild (V_2O) has conferred to cocoa seedlings a moderate resistance against *P. megakarya*; here weblike patches (3) were prominent on leaf disks compared with penetration points (1) and connected points (2).

The Mycorrhizae-based treatment (V_2M) and organic fertilizer combinations with Mycorrhizae (V_2MO) or *T. asperellum* strain PR11 (V_2TO) are less efficient in the variety SNK 13 and cause the susceptibility of young plants of this variety to *P. megakarya*. The infection severity index here is up to 2.5 and then conferred to leaf disks the presence of symptoms from scales 2, 3 and 4. Finally, the presence of treatment $V_2MOTNPK$, V_2MT,

Table 3. Categorization of treatments depending on the degree of susceptibility of V_2 to *P. megakarya.*

Treatments	Infection Severity Index	Degree of susceptibility
V_2MOT	1.27	Resistant
V_2T	1.55	
V_2O	2.27	Moderatly resistant
V_2M	2.69	
V_2TO	2.72	Susceptible
V_2MO	3.45	
V_2MOTNPK	3.55	
V_2MT	3.69	
V_2NPKR	4.66	Highly susceptible
V_2TS	4.83	
V_2T-	4.86	

V_2NPKR, V_2T- and V_2TS do not prevent the seedlings of cocoa correspondent that are highly susceptible to infection. Leaf disks from these treatments were affected generally by mottle patch (4) and true necrosis (5) and then increased the infection index.

DISCUSSION

The results confirm the good response of leaf disks of seedlings of the variety Tafo 79/501 issued from treatments V_1MOT, V_1T, V_1TO, V_1O, V_1MO, V_1M, V_1MT and V_1MOTNPK compared with the variety SNK 13 where only V_2MOT, V_2T and V_2O showed resistance against *P. megakarya*. Our results corroborated those from Blaha and Lotodé (1976) which classified the variety Tafo 79/501 ahead of SNK 13 in terms of their ability to tolerate the pathogen of cocoa black pod disease, *P. megakarya*.

The results also show the good behavior of the leaf disks derived from plants inoculated by the two biofertilizers namely PR11 and AMF, including very good results in the case of isolated inoculations as dualistic. Our results are in agreement with those of several authors who have conducted research works on the effect of both biofertilizers on resistance *in vitro* of seedlings of cocoa against *P. megakarya* using the techniques of leaf disks (Nyassé et al. 1995; Tahi et al., 2006; Tondje et al., 2007; Tchameni et al., 2011).

Furthermore, research carried out by Paulitz and Belanger (2001), Harrier and Watson (2004), Jemo et al. (2007), Tondje et al. (2007), Nwaga et al. (2007, 2010) and Tchameni et al. (2011) showed that AMF and members of the genus *Trichoderma* have emerged as promising groups of microbial inoculants that can induce plant growth and resistance to diseases. Indeed, the roots of more than 80% of plant species are generally colonized by the AMF which are beneficial in combination with their host (Harley and Smith, 1983).

According to Sikes et al. (2009), AMFs have a protective effect against some plant pathogens. The results of Harrier and Watson (2004) found that colonization of roots of cocoa by the AMF reduces the susceptibility of the crop to 50-70% of diseases; in the same order, Nwaga et al. (2007, 2010) have shown that the action of the fungi on the host plant reduces root and foliar diseases caused by pathogens.

As well, the fungi of the genus *Trichoderma* are commonly used in the biocontrol of diseases that affect plant species (Jemo et al., 2007; Vinale et al., 2008). In addition, *Trichoderma* spp. in their interactions with plant roots provide nutrients to their hosts, promote the growth of these, increase their productivity and improve their disease resistance capacity (Martinez et al., 2001; Harman, 2008; Tchameni et al., 2011).

The combination of the two strains of Mycorrhizae namely *Gigaspora margarita* and *Acaulospora tuberculata* with the strain PR 11 of *Trichoderma asperellum* (V_1MT and V_2MT) give unsatisfactory results than when each biofertilizer is inoculated individually. The study results agree with those on cocoa seedlings in the nursery by Tondje et al. (2007).

According to these authors, the dualism between mycorrhizae and *Trichoderma* fungi causes an antagonistic effect between the two types of biofertilizers. Active agents of mycoparasitism, it is possible in the association that, the PR 11 strain of *T. asperellum* develops a negative interference effect with mycorrhizae strains used, as suggested by Rousseau et al. (1996). Similar results within the antagonism between the two biofertilizers were also found on bean (Martinez et al., 2001).

Indeed, the ability of dual inoculation with AMF and *T. asperellum* to enhance growth and induce systemic resistance of seedlings was less functional, showing their possible incompatibility to occupy the same rhizosphere (Tchameni et al., 2011).

However, organic fertilizer (Gro-wild) gave interesting results in terms of protection of cocoa seedlings against *P. megakarya*. Indeed, according to Cantin (2001), organic fertilizers stimulate soil biological activity. Because of their ability to inoculate thousands of microorganisms in the soil and increase their biodiversity, organic fertilizers contribute to phenomena such as plant protection against certain fungal infections and bacterial in nature (Cantin, 2001).

Furthermore, it is known that organic fertilizers are sources of nutrients for microorganisms in the soil (Cantin, 2001). It is therefore possible to think that the presence of Gro-wild in the dualism between Mycorrhizae and *Trichoderma asperellum* strains PR11 (V_1MOT and V_2MOT) would be the cause of a synergistic action of the two microorganisms; that would explain why these combinations provide treatment more efficiency to both cocoa varieties studied.

Conclusion

The main objective was to evaluate *in vitro* resistance of cocoa seedlings of both varieties Tafo 79/501 and SNK 13 to the agent of black pod disease *P. megakarya*. Inoculation of biofertilizers in experimental pots gave good protection of cocoa seedlings against the development of *P. megakarya*. The latter confirmed their status as crop protection microorganisms. However, their effectiveness was increased in their association with organic fertilizer Gro-wild. Cocoa farmers can benefit from the use of biofertilizers like the AMF and PR11 strains of *T. asperellum* instead of the usual pesticides; indeed, the foliar resistance of cocoa to *Phytophthora* spp. is an indicator of the resistance of the pods in the field.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Genetic divergence in *Agave* accessions through ISSR markers and phenotypic traits

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The *Agave* genus is composed of about 200 species, but the cultivation of sisal for fiber production in Brazil is restricted to two species: *Agave sisalana* and *Agave fourcroydes,* both have several accessions with wide variability. The collection of *Agave* of Embrapa has 37 accessions maintained *in situ* and periodically evaluated agronomical traits. Most of these accessions have phenotypic similarities, although they differ in fiber quality, which are widely used for commercial purposes. The identification of promising accesses contributes to the advance in improvement works, focusing on commercial indication. In order to estimate the genetic divergence of this collection, a cluster analyses was performed based on Inter-Simple Sequence Repeat (ISSR) markers and phenotypic traits. Genomic DNA from these accessions were used in polymerase chain reaction (PCR) with thirty ISSR oligonucleotides. For phenotypic characterization, twelve descriptors were adopted based on morphological and agronomic data. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Tocher multivariate methods were adopted. Thirteen groups were formed by the Tocher Method and six by UPGMA; however UPGMA method was more representative in the group formation. The comparison of the band patterns among accessions derived from the shoots showed that genetic variability is generated during asexual reproduction in these plants. The four lines generated from Tatui were the most divergent accessions. These plants are tallest, with higher mass values of fresh and dry mucilage, fresh and dry fiber mass, fiber length and presence of spines at the edges. The accessions from Instituto Agronômico de Campinas (IAC) showed the lowest genetic distances, indicating a possible narrow genetic base and high kinship degree. The crossings between H-RN, H-Kenya, H-400 fls, and H-11648 with Tatuí 1, 2, 3, and 4 can be a valorous strategy to broadening genetic diversity among commercial and native sisal germplasm.

Key words: Sisal, *Agave sisalana,* molecular markers, morphoagronomic, diversity, cropping breeding.

INTRODUCTION

Agave is a xerophytic and monocot plant that grows natively in semi-arid, subtropical, and tropical regions from the southern United States to northern South America and throughout the Caribbean (Infante et al., 2006). Based on Angiosperm Phylogeny Group (APG) III, Bremer et al. (2009) cited the new classification for the genus *Agave*, which became part of the Asparragaceae family. The genus *Agave,* which originated from arid and semi-arid regions of Mexico, contains about 200 species, with restricted geographic distribution (Gentry, 1982; Garcıa-Mendoza and Galvan, 1995). *Agave sisalana* Perr. and *Agave fourcroydes* (henequen) are broadly cultivated due to their fiber qualities (Judd et al., 2007; Martin et al., 2009). The reproductive mechanisms of most agaves involve reproduction by seeds and through rhizomes, which appear in early stages of plant development, forming new individuals (Infante et al., 2006). The plant has hermaphrodite flowers and dehiscent fruits. The flowering takes place only once, during the whole cycle. The fiber is the hardest among the fibrous species and has wide use in the artisanal segment, civil construction and automobile industry (Soto et al., 2013). In addition, the mucilage derived from defibration process can be used in ruminant feeding (Silva et al., 1999; Brandão et al., 2011; Macedo et al., 2015).

Brazil is the world's largest producer and exporter of thread and manufacturer of sisal (*A. sisalana*) (CONAB, 2016). It is an important crop in semi-arid region, where total rainfall is often scarce and irregular. Despite the widespread use of sisal, information about the genetic basis of genotypes grown in the Northeast region is limited. It is assumed that the cultivars must have the same genetic basis, although *A. sisalana* is not a genetically pure germplasm (Lock, 1962). According to Moreira and Vieira (1999), the lack of knowledge on heritability of the fiber-related traits has limited the progress of sisal improvement, although they report that the resistance and fiber percentage have high heritability, and therefore can provide selection gains in breeding program. The Brazilian Agricultural Research Corporation (Embrapa) has an *Agave* collection, containing *A. sisalana* and *A. fourcroydes* accessions, maintained *in vivo* in a semi-arid environment, in the Cariri region of Paraíba, Brazil. Phenotypic descriptors, based on morphological and agronomic traits are periodically recorded. Knowledge of the genetic diversity of the collection is limited, although such information is essential to estimate the potential of the genotypes for later use in breeding program. The analysis of genetic divergence in germplasm banks provides several useful information on genetic resources, based on a data set. The multivariate methods are important tools to assist the selection procedures in improvement programs and provide broad contribution to classification and identification of germplasm. Among the most used multivariate techniques, the unweighted paired group method using arithmetic averages (UPGMA) is widely adopted by breeders because it is consistent with regard to the allocation of clusters. When different types and number of traits were used, it also revealed higher cophenetic correlation coefficient (Sneath and Sokal, 1973). Other widely used method is the Tocher's optimization method that uses the criterion of optimization, minimizing the average distance intra-cluster and maximizing the average distance inter-cluster (Rao, 1952). However, the Tocher's method does not involve a construction of a phenogram to perform the clustering. The clustering is normally used together with UPGMA, revealing correspondence to the allocation of elements in the groups (Arriel et al., 2006).

Although studies involving genetic diversity in sisal collections are limited, some findings are reported in the literature. Navarro-Quezada et al. (2003) analyzed the genetic differentiation of *A. deserti* complex, comprising *A. deserti*, *A. cerulata* and *A. subsimplex*, using Nei's genetic distance from random amplified polymorphic DNA (RAPD) markers. According to authors, Nei's genetic distances between the three species were low compared to the values obtained from other Agavaceae, and there was no clear correlation with taxonomic divisions. In an UPGMA analysis, *A. subsimplex* and *A. cerulata* formed exclusive monospecific clusters, whereas the *A. deserti* populations appeared in more than one cluster together with other species. The results were consistent with a pattern of genetic isolation by distance. With the Tocher method, Moreira and Vieira (1999) used agronomic traits collected in seven accessions to estimate the genetic divergence, based on agronomic data. Despite the reduced number of accessions, the authors were able to discriminate them in five groups, with 11648 and IAC 069 being the most divergent and therefore those were recommended for later use in breeding program. The perspective of acquiring heterotic expression with these genotypes is high, considering the genetic base of the genotype 11648 which is an interspecific hybrid between *A. angustifolia* × *A. amaniensis* (Doughty, 1938).

The *Agave* collection of Embrapa has been periodically evaluated to agronomical traits and some biochemical studies have been carried out as to mucilage proprieties of *A. sisalana* accessions (Oliveira et al., 2016). Based on evaluations carried out by Moreira and Vieira (1999), most accessions have phenotypic similarities, although they differ in some fiber traits. The identification of promising accesses is relevant to improvement of sisal, because it contributes to further indication of commercial genotypes.

The aim of this work is to determinate the genetic

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divergence among the *Agave* accessions from Embrapa performed by a cluster analysis using UPGMA and Tocher methods, based on ISSR markers and phenotypic traits.

MATERIALS AND METHODS

Genetic resources and ISRR-PCR assays

In this study, 37 accessions of the sisal collection were used, maintained in Monteiro, PB, Brazil (07°53'22 "S, 37°07'40" W, 599 m). A summary of the phenotypic data of the accessions is found in Table 1.

Fresh leaves of each accession were used for DNA extraction based on CTAB method (Ferreira and Grattapaglia, 1998). The PCR assays were performed in a Mastercycler Gradient (Amplitherm Thermal Cyclers). The reaction mixture (25 µl) contained 10× PCR buffer (Fermentas), 25 mM $MgCl_2$, 100 mM dNTP, 1.2 $\mu Mol.L^{-1}$ of each ISSR primer (USB), 60 ng of genomic DNA, and 1 U of Taq DNA polymerase (Ludwig Biotec). Ten ISSR oligonucleotides were used to PCR assays. The samples were submitted to follow the program: initial cycle of denaturation at 95°C/5 min, followed by 40 cycles of denaturation at 95°C/1 min, annealing at 50°C/1 min, and extension at 72°C/2 min. A final extension cycle was added to reaction at 72°C/7 min. The amplified products were analyzed by electrophoresis on 2.5% agarose gel. All reactions were performed in triplicate.

Analysis of genetic divergence and clustering techniques

For the multivariate analysis, molecular and phenotypic data were used. In the molecular data, each band generated by ISSR-PCR was coded with 1 and 0 for presence and absence, respectively, resulting in a binary matrix, which was used to quantify the genetic similarity (Sij) among the accession pairs based on the coefficient of Jaccard, following the expression:

$$S_{ij} = \frac{a}{a} + b + c$$

Where: *a* means the sum of coincidences of type 1-1 for each pair of accession, *b*, the sum of the discordances of type 1-0 for each pair of accession, and *c*, the sum of discordances of type 0-1 for each pair of accession. The arithmetical complement 1- S_{ij} was also used to transform the similarity matrix into a dissimilarity matrix, which is used in cluster analysis. The estimated dissimilarity matrix for the phenotypic data was obtained based on the Gower distance (Gower, 1971), allowing the treatment of phenotypic data simultaneously. The sum of the two matrices was performed to obtain a representative matrix of the molecular and phenotypic data, which was used to perform the cluster analysis. The dissimilarity values of each matrix were standardized through the expression:

$$d_{pj} = \frac{d_{ij}}{\sigma d}$$

Where, d_{pj} is the standardized dissimilarity between an individual *i* and individual *j*; d_{ij} is the dissimilarity between *i* and *j*, and σ_d is the standard deviation of dissimilarity.

The correlation between the matrices was also made with the purpose of estimating the level of relation between them, obtaining the significance from the t and Mantel tests, indicating or not, the agreement of the two methods in expressing the existing genetic difference between pairs of accessions (Cruz, 2008).

The clustering analyses were carried out through Tocher's optimization and UPGMA methods. The cophenetic correlation coefficient was estimated in order to eliminate the non-hierarchical effects. Cluster analysis was performed using the software GENES, version 2016.6.0 (Cruz, 2013). The following phenotypical traits were used for analysis: number of leaves, plant height, leaf length, fresh leaf mass, dry mucilage mass, fresh fiber mass, dry fiber mass, fiber length, presence of spines on the leaf edge, tillering and high folding endurance.

RESULTS AND DISCUSSION

Phenotypic traits (Table 1) and ISSR markers were used to estimate the genetic divergence among thirty-seven sisal accessions, maintained *in situ*, in the semi-arid zone Cariri of Paraíba, Brazil (Figure 1A-F). Adult plants were used for agronomical characterization, with subsequent molecular analysis, using leaf tissues.

In order to optimize molecular analysis, thirty oligonucleotides were previously tested for polymorphic selection, and therefore contributory to the evaluation processes. Of the total, ten oligos were selected, with high polymorphism rate, revealed by amplicons obtained from agarose gels, with an average of 13/oligo (Table 2). Although we used a relatively small population, maintained asexually, it was possible to identify differences among accessions through profiles obtained via ISSR-PCR. Several bands were common among the accessions; however, some were unique and therefore contributory to the analysis of genetic divergence of the collection. Figure 1G shows a pattern of amplicons obtained with UBC 812, with 11 bands distributed between 0.1 and 1 Kb.

Most molecular markers are robust to identify variability in sexually or asexually propagated populations. In literature, ISSR, amplified fragment length polymorphism (AFLP) and microsatellites markers are the most reported (Barraza-Morales et al., 2006; Abraham-Juárez et al., 2009; Santos et al., 2015). In *Agave*, the production is supported by intensive clonal propagation and the suppression of the sexual reproduction, leading to reduction in genetic variability plantations (Gil-Vega et al., 2001; Infante et al., 2006; Abraham-Juárez et al., 2009).

Abraham-Juárez et al. (2009) analyzed the genetic variability by AFLP markers, using three reproductive forms of *A. tequilana*, and found 75.08 and 86.06% of polymorphic loci, respectively, from offsets and bulbils generated from the same matrix plant. As to authors, although a significant level of polymorphism was observed between rhizome offsets, the levels were even higher between bulbils, reaching levels comparable to those found between plantlets produced from seeds (90.1%). Infante et al. (2006) explained the origin of the asexual variability in *Agave* species based on molecular markers. According to authors, in plant shoots, the whole cells are derived from the apical meristem, including the germline cells. In asexual reproduction, meristems are the source of the mitotically derived offprints. The only source of genetic differences in these materials comes

Table 1. Phenotypic traits of the sisal collection, based on an average of 3 years.

Accession	Name	NL	PH	LL	FLM	FMM	DMM	FFM	DFM	FL	PS	NT	HFE
SS-01	H-11648	69.3	104.8	77.1	283.6	220.4	26.8	22.5	10.6	76.5	2	3	2
SS-02	H-400 fls	75. 6	151.1	89.1	304.6	199.3	25.2	31.5	10.6	84.5	2	3	2
SS-03	Cabinho	34.6	170.0	111.2	604.1	317.3	38.5	79.7	28.2	109.8	1	2	1
SS-04	IAC 034	36.0	133.3	86.5	306.3	178.5	19.9	66.4	19.0	91.3	2	3	2
SS-05	H-RN	65.0	124.2	84.7	306.6	168.5	23.1	31.7	18.8	82.1	2	3	2
SS-06	H-Quênia	62.8	147.0	104.9	428.5	162.1	20.1	59.1	15.2	77.5	2	3	2
SS-07	*A. fourcroydes*	33.3	162.5	97.9	686.9	392.3	47.5	80.1	27.3	109.5	1	2	2
SS-08	Tatuí	17.3	117.1	67.2	895.5	477.2	51.3	63.8	18.5	99.5	1	2	1
SS-09	Espinho	27.8	152.0	89.9	303.1	132.9	24.3	35.8	14.0	90.7	1	3	1
SS-10	Valente	29.3	112.0	70.6	318.6	230.7	25.9	27.3	11.4	88.5	1	2	2
SS-11	H-Teixeira	57.2	126.6	91.9	276.0	196.0	18.6	31.2	11.4	74.6	1	3	2
SS-12	H-Imaculada	66.3	145.6	106.0	392.2	160.7	21.6	31.2	15.0	89.8	2	3	2
SS-13	Ornamental	25.0	98.5	61.7	135.6	83.7	12.2	19.5	6.8	61.1	1	1	2
SS-14	Tatuí 3	27.0	183.5	105.2	855.6	479.8	47.6	107.6	29.3	131.2	1	3	1
SS-15	Tatuí 4	33.2	187.8	110.5	1588.2	904.1	92.4	146.5	45.5	132.8	1	3	1
SS-16	Mutante 1	38.0	149.2	93.6	521.9	269.6	30.9	45.5	20.5	96.1	1	3	1
SS-17	Hoxa México	35.0	135.3	85.1	364.0	200.6	25.3	41.2	17.0	88.3	2	3	2
SS-18	Tatuí 1	32.0	179.0	105.5	1419.6	853.5	82.1	142.1	39.1	122.7	1	3	1
SS-19	Tatuí 2	19.5	153.0	86.2	670.1	322.2	38.3	102.5	28.5	112.1	1	3	1
SS-20	Tanzânia	49.5	133.5	91.5	342.3	147.2	18.3	29.6	13.6	82.6	2	3	2
SS-21	Mutante PB	26.8	124.8	75.8	357.8	209.4	21.6	38.7	17.1	86.0	1	3	1
SS-22	IAC 0101	27.6	106.6	67.1	204.6	140.7	15.8	45.8	10.8	73.1	2	2	2
SS-23	IAC 84193	19.6	80.4	50.0	115.2	82.8	8.4	34.9	6.9	58.5	2	1	2
SS-24	Mutante BA	31.1	133.5	82.3	409.5	179.7	17.3	41.1	18.5	87.1	2	2	2
SS-25	IAC 84003	26.0	121.5	73.7	274.5	243.3	27.6	46.3	17.3	94.5	2	3	2
SS-26	IAC 84051	34.8	94.6	64.7	191.1	152.6	20.9	30.0	11.7	65.8	2	2	2
SS-27	IAC 00200	29.0	93.2	61.1	267.0	142.2	18.5	42.8	16.1	74.1	2	2	2
SS-28	IAC 0067	44.0	129.0	86.5	299.5	230.4	33.9	35.7	15.9	90.5	2	2	2
SS-29	IAC 84001/4	38.0	104.0	71.0	338.9	190.7	19.8	29.8	11.6	83.0	2	3	2
SS-30	IAC 84005	50.6	134.4	92.5	265.1	229.7	44.8	42.3	16.7	79.6	2	3	2
SS-31	IAC 0056	36.0	98.8	67.4	374.3	121.1	16.5	30.9	9.8	73.5	2	3	2
SS-32	84001/2	38.0	104.0	71.0	218.4	115.4	16.6	32.6	13.4	70.1	2	3	2
SS-33	IAC 84-019	44.3	107.5	75.9	241.3	153.2	23.9	69.1	27.1	88.0	2	1	2
SS-34	IAC 840096	33.3	89.0	61.1	126.6	65.0	8.5	23.2	7.9	60.6	2	2	2
SS-35	IAC 0097	28.4	105.8	67.1	140.8	59.4	9.3	34.7	12.8	73.4	2	1	2
SS-36	IAC 0069	30.0	101.0	65.5	110.3	57.0	9.2	13.9	5.4	64.2	2	3	2
SS-37	H- Itaporanga	51.0	126.7	88.8	293.2	260.7	22.0	22.1	9.9	76.5	2	3	2

H- hybrid; number of leaves (NL); height of the plant from the base to the crown (PH); length of fully expanded mature leaf (LL); fresh leaf mass (FLM); fresh mucilage mass (FMM); dry mucilage mass (DMM); fresh fiber mass (FFM); dry fiber mass (DFM); fiber length (FL); presence of spines at the edges (PS): 1 = has spines, 2 = has no spines; number of tiller (NT): 1 = without tiller, 2 = between 4 and 5 tiller, 3 = ≤ 5 tiller; high folding endurance (HFE): 1 = no resistance, 2 = resistant.

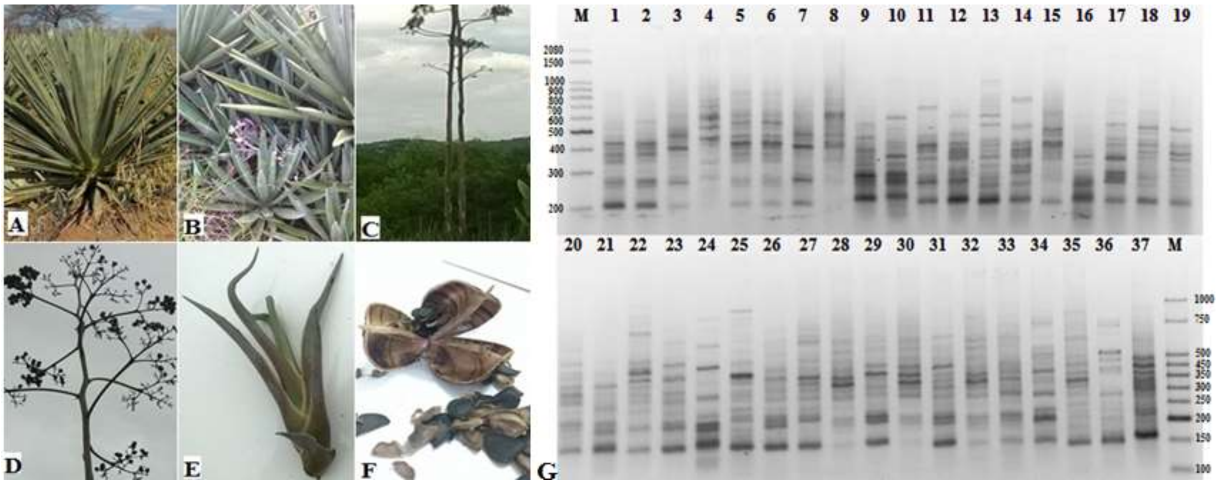

Figure 1. Details of a sisal accession, maintained in the Cariri environment (Monteiro-PB, Brazil). A- Adult plant, B- Tiller originated from the matrix plant, C- Floral scape, D- Panicle with bunch flowers, E- Bulbils located at floral scape, F- Mature capsule and round-triangular seeds, G- Amplicons obtained by ISSR-PCR, using UBC 812 oligonucleotide. M- Molecular marker (Ludwig Biotec). List of accessions in Table 1.

Table 2. Sequence of ISSR oligonucleotides used in the genetic analysis of sisal accessions and total number of bands (TNB) obtained by ISSR-PCR.

Oligonucleotides	Sequence $(5' \rightarrow 3')$	TNB
UBC 808	AGA GAG AGA GAG AGA GC	19
UBC 809	AGA GAG AGA GAG AGA GG	15
UBC 812	GAG AGA GAG AGA GAG AA	11
UBC 823	TCT CTC TCT CTC TCT CC	11
UBC 824	TCT CTC TCT CTC TCT CG	8
UBC 825	ACA CAC ACA CAC ACA CT	13
UBC 827	ACA CAC ACA CAC ACA CG	11
UBC 830	TGT GTG TGT GTG TGT GG	12
UBC 853	TCT CTC TCT CTC TCT CRT	12
UBC 881	GGG TGG GGT GGG GTG	11

from somatic mutations.

Although the *Agave* has both forms of propagation, the sexual process that is responsible for segregation and high variability is not frequent and only occurs under special conditions, such as when the tassel is decapitated before the emission of floriferous branches leading the plants to produce viable seeds and fruits. However, it is a time-consuming process and when it occurs, the progenies often show marginal leaf spines, an undesirable trait for clones destined for commercial exploitation (Abraham-Juárez et al., 2009).

The clustering analyses by Tocher and UPGMA are found in Table 3 and Figure 2, respectively. Through Tocher method, 13 groups were formed, of which 6 had only one accession. The group 1 contained the majority of accessions (40.5%), with medium height and high number of leaves. Among them, stands out the hybrids RN, Kenya, 400 fls, 11648 and *A. sisalana*, all established in Brazilian semi-arid region. According to Silva et al. (2008), the hybrid 11648 and *A. sisalana* are widely grown in Northeast region, due to acceptable traits to fiber market. The group 2 contained accessions with high height and long fibers, represented by Cabinho and *A. fourcroydes*. The group 3 clustered the accessions from the Instituto Agronômico de Campinas (IAC 840096, IAC 0069, IAC 0097, IAC 0101) and an ornamental accession (ornamental Sisalana). These materials have medium and short fibers and have high folding endurance. The group 4 contained two IAC accessions (IAC 84003, IAC 84001/4), all showing low number of leaves and no spines at the edges. The group 5 grouped three lines from Tatuí, all of them are tall plants, with greater leaf weight and extra-long fibers. These attributes are interesting for further use in breeding work. The groups 6 and 7 clustered two accessions each, with tall plants, medium fibers, spines at the edges and tillering, but those of group 6 (Valente, Teixeira) had high folding endurance, which does not occur in group 7 (Espinho, Mutant Paraíba).

In clustering analysis via UPGMA, the number of

Table 3. Grouping of sisal accessions by Tocher optimization method, based on phenotypic and molecular data.

Groups	Accessions
1	H-RN, H-Quênia, H-400 folhas, H-11648, IAC 84005, IAC 0067, 84001/2, Sisalana Tanzânia, IAC 0056, H-Itaporanga, IAC 00200, H-Imaculada, Hoxa México, IAC 84051, IAC 84-019
2	Cabinho, *A. fourcroydes*
3	IAC 840096, IAC 0069, IAC 0097, Sisalana ornamental, IAC 0101
4	IAC 84003, IAC 84001/4
5	Tatuí 4, Tatuí 1, Tatuí 3
6	Valente, Teixeira
7	Espinho, Mutante Paraíba
8	Mutante 1
9	IAC 034
10	Mutante Bahia
11	Tatuí 2
12	IAC 84193
13	Tatuí

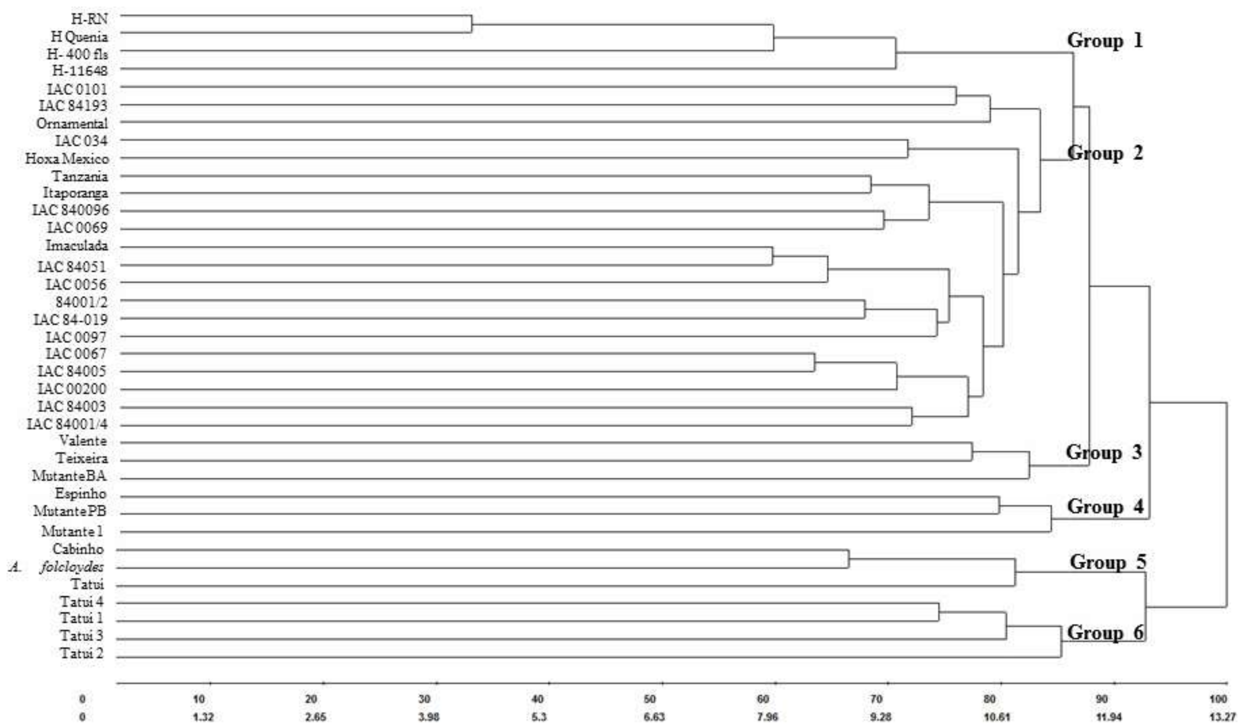

Figure 2. Dendrogram obtained by UPGMA method, generated from a similarity matrix with 37 agave genotypes. Cophenetic correlation coefficient: 0.77. A similarity index up to 85% was adopted ($p\leq0.01$, F test).

grouping was more condensed, distributed in six clusters. The degree of association between dissimilarity matrices obtained from phenotypic and molecular data was significant, based on t and Mantel test ($p\leq1\%$), indicating that both sets of data were adequate to represent the genetic divergence in the collection of sisal. The distribution of accessions through UPGMA was more contributive to discriminate divergent groups than Tocher's and provides more possibilities for choice of parents in breeding work aiming at production and fiber quality. UPGMA method tends to generate higher values of the cophenetic correlation coefficient, allowing to infer that the groups of accessions indicated in the graphic have an adequate fit between the dissimilarity matrix and dendogram obtained (Cruz and Carneiro, 2003).

As shown in Figure 2, the hybrids H-400 fls, H-Kenya, H-RN, and H-11648 were clustered in a group separated from the others. All of them are bred genotypes, with special traits to the natural fiber market. Overall, the fiber of these hybrids shows strength and yield near to 750 g/force and 2.7 $t.ha^{-1}$, respectively (Amorim Neto and Beltrão, 1999). The most IAC accessions were clustered in group 2 that contained 20 accessions, and most showed short fiber. The groups 3 and 4 contained three accessions each, constituted by mutants collated in Paraíba and Bahia states, and also local types. The group 5 contained *A. fourcroydes*, Cabinho and Tatuí, all characterized by presence of spines at the leaf edges. The last, group 6, clustered the four lines derived from Tatuí, characterized by high weigh of leaf (fresh mass) and dry mucilage, high weigh of fiber (dry and fresh), large fiber length and presence of spines at the leaf edges. They are similar lines, spreading in different places in Sao Paulo State, located at Sudeste region, Brazil.

Based on results obtained from clustering analysis, we can consider that the variability found in the Embrapa-*Agave* collection offers possibilities to implement a breeding program in order to meet the demands of the natural fiber market. The richness of this information lies in the fact that, although *Agave* is originated from Mexico, Brazil has several ecotypes that have as their main skill the broad adaptation to the semi-arid and tropical environments. There is no information on the existence of variability in fiber quality in different environments. However, in the literature, the phenotypical differences in plant canopy are marked when grown in less arid environments (Silva et al., 2008). Thus, depending on the purpose of the breeding program, it is possible to identify in these results divergent genotypes that may contribute to the generation of promising hybrids.

CONCLUSION

Agronomical and molecular variability was found in *Agave* collection, maintained by Embrapa, in Monteiro, PB, Brazil. This represent a valorous strategy to broadening genetic diversity among commercial and native sisal germplasm.

CONFLICT OF INTERESTS

The authors declare no conflict of interest between the partners with the release the results.

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