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Effect of cover crops on the suppressiveness of root rot and on the agronomic characteristics of cassava

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Abstract

Cassava (*Manihot esculenta* Crantz) is a major staple food in many developing countries, including Brazil. However, it faces significant challenges from diseases like dry root rot (DRR) and black root rot (BRR) caused by soil-borne fungi. This study explores the use of cover crops to suppress these diseases and improve cassava's agronomic traits. Over four cultivation cycles, various cover crops were alternated with cassava varieties 'BRS Kiriris' (resistant) and 'BRS Formosa' (susceptible) in soil infested with DRR and BRR pathogens. In the 2nd cycle, only germination showed significant differences, but by the 4th cycle, various agronomic parameters, including fresh weight (FW.AP), dry weight (DW.AP) of the aerial part, fresh weight (FW.Root), dry weight (DW.Root) of the roots, and plant vigor, exhibited significant improvements. Cowpeas were identified as the most effective cover crop in disease reduction (ID%) and promoting healthier and taller cassava plants. Jack beans and peanuts also had positive effects on vigor, FW.AP, DW.AP, FW.root, and DW.root. In contrast, cover crops like maize, black oats, *Crotalaria ochroleuca*, cassava, and fallow soil had minimal impacts. Throughout the cycles, these selected cover crops consistently contributed to disease reduction and improved cassava's agronomic characteristics. This research highlights their potential in holistic disease management strategies.

Keywords: soil management, crop succession, cassava root rot, disease suppression

1. Introduction

Cassava (*Manihot esculenta* Crantz) is one of the crops with great economic and social potential for developing countries in tropical and subtropical areas (Silva et al., 2017), it is a staple food for almost one billion people in 105 countries, for which it is considered one of the food safety cultures for the world. Being the third largest source of carbohydrates in the tropics, behind only rice and maize. In recent years, it has been growing in industrial applications such as biofuels, biopolymers, food, feed, cosmetics and medicine (Li et al., 2017; Mombo et al., 2017).

Among the main producing countries, Brazil stands out as the sixth largest producer of cassava root in the world, with a production of 18.2 million tons (FAO, 2022) reach 80% of production (Brito et al., 2020). Root rot can be separated into at least three types, depending on the symptoms presented and the pathogenic agents involved, such as dry rot, usually associated with fungi of the genus *Fusarium* and the species *Macrophomina pseudophaseolina*, (Onyeka et al., 2005; Boas et al., 2016; Hohenfeld et al., 2018); black rot, whose main agents involved belong to the genus *Lasiodiplodia* and *Neoscytalidium dimidiatum* and soft rot, associated with oomycetes of the genera *Phytophthora* spp., *Phytophthora* spp. and *Pythium* spp. (Machado et al., 2014; Hohenfeld et al., 2018; Brito et al., 2020).

These diseases are caused by soil-borne pathogens with great saprophytic and survival capacity, which is why they are difficult to manage diseases (Machado et al., 2018). Thus, it is essential to adopt strategies aimed at reducing the primary inoculum and providing better agronomic characteristics of the plants.

The use of resistant varieties is the most viable and economical strategy for controlling the disease, but the development of these varieties is difficult, because these pathogens survive in the soil and the appearance of symptoms can be influenced by climatic conditions and the soil (Santiago et al., al. 2018). The use of chemical control has not yet been validated for the management of cassava root rot, and when applied improperly, it can have a deleterious effect on the environment (Machado et al., 2018).

Alternatively, the induction of suppressiveness is a promising strategy, given the positive results obtained in recent literature (Barros et al., 2014; Silva et al., 2017; De Medeiros et al., 2019). This suppressiveness of the soil consists of limiting the disease, because despite adequate conditions such as a susceptible plant, virulent pathogen and an environment that favors the development of the disease, the disease may not occur, but if it does, it would be less severe (Bettiol and Ghini 2005; Kinkel et al., 2011).

The induction of suppressiveness is related to the biotic and abiotic factors of the soil, the plant and the environment, the set of these factors may imply the suppressiveness or conductivity of the disease (Garbeva et al., 2004; Bonanomi et al., 2007). Covering crops such as legumes, jack beans (*Canavalia ensiformis*), cowpea (*Vigna Unguiculata*), crotalaria (*Crotalaria Ochroleuca*), peanuts (*Arachis hypogaea*), have a low C/N ratio, have the ability to use phosphorus (P) and potassium (K) in fractions that are almost inaccessible in the soil, improving P and K availability for subsequent crops (Fisher et al., 1999; Mitran et al. 2018; Hallama et al. 2019). While moonocots such as maize (*Zea Mays*) and black oats (*Avena strigosa*), due to their high C/N ratio, can stimulate microbial activity, reducing nitrogen availability, consequently impairing the pathogen's infection process (Donofrio et al. 2006; Bonanomi et al. 2007; Sun et al. 2020).

Some cover crops have the ability to induce soil suppressiveness, however there are still several factors that limit their use, as the impact of this management on pathogen populations and disease suppression is still unpredictable. Reliable guidelines for predicting the impact of any type of organic matter alteration on specific soil diseases are lacking (Bonanomi et al., 2007). Therefore, the objectives of this study were: (i) to evaluate the effect of different types of cover crops incorporated into the soil in the suppression of dry and black root rot in cassava; (ii) characterize the effects of different types of cover crops incorporated into the soil on the agronomic characteristics of cassava.

2. Material and methods

The experiments were carried out in a greenhouse with a temperature of 32 ± 2 °C at Embrapa Mandioca e Fruticultura, in the municipality of Cruz das Almas (Bahia, Brazil). The soil came from the experimental area of Embrapa Tabuleiros Costeiros (11°22'37.9"S, 37°40'29.6"W), located in the municipality of Umbaúba/SE, with a history of high incidence of cassava root rot. The soil was classified as a typical dystrophic cohesive Yellow Oxisol, with a sandy-clay-loam texture and the following chemical characteristics: **pH** (H₂O)= 5.14; **P** (mg/dm³)= 50.8; **K** (mg/dm³): = 235; **V** (%) = 65; **m** (%): 0; **MO** (dag/kg) = 2.69.

This work consisted of two independent experiments that were set up 15 days apart, however the last evaluation was conducted at the same time, which generates different cycle durations, being one trial harvested 75 days after planting (DAP), while the other trial 90 DAP. For each of the experiments, four cultivation cycles were carried out with 11 treatments of different combinations of fallow (no cultivation),

cassava varieties and cover crops: *Crotalaria ochroleuca*, black oat (*Avena strigosa*), jack bean (*Canavalia ensiformis*), maize (*Zea mays*), peanut (*Arachis hypogea*) and cowpea (*Vigna unguiculata*) (Table 1). The experimental design was in randomized blocks (RBD), in a 11x2 factorial scheme, with 11 combinations of cover crops, cassava and fallow × two varieties of cassava ('BRS Kiriris' and 'BRS Formosa'), with four blocks, with a plot consisting of three pots with two plants each.

Table 1 Treatments and crops used to assess the potential of cover crops to induce suppressiveness of cassava root rot during cropping cycles.

Treatment	Cultivation			
	1 st	2 nd	3 rd	4 th
1	Fallow	Fallow	Fallow	Cassava
2	Fallow	Fallow	Cassava	Cassava
3	Fallow	Cassava	Cassava	Cassava
4	Fallow	Cassava	Fallow	Cassava
5	Cowpea	Cassava	Cowpea	Cassava
6	Jack bean	Cassava	Jack bean	Cassava
7	Crotalaria	Cassava	Crotalaria	Cassava
8	Peanut	Cassava	Peanut	Cassava
9	Maize	Cassava	Maize	Cassava
10	Black oat	Cassava	Black oat	Cassava
11	Cassava	Cassava	Cassava	Cassava

Fallow = without cover crop; Cassava (*Manihot esculenta*); Cowpea (*Vigna unguiculata*); Jack bean; Maize (*Zea mays*), Crotalaria (*Crotalaria ochroleuca*); Black oats (*Avena strigosa*); Peanut (*Arachis hypogea*).

2.1 Inoculum preparation and soil infestation

Isolates of pathogens that cause dry rot (*Fusarium oxysporum* (CBPPR0006)/ KT211499, *F. equiseti* (CBPPR0031)/KT211523, *Fusarium verticillioides* (CBPPR0048)/ KT211540, *Fusarium solani* (CBPPR0029)/ KT211521, *Fusarium lateritium* (CBPPR0012)/ KT211505 and *F. chlamydosporum* (CBPPR0008)/ KT211501 and black rot (*Lasiodiplodia theobromae* (CBPPR1002)/ KT211551 and *Neoscytalidium dimidiatum* (CBPPR1011)/ KT211560 all from the mycotheque of the Phytopathology Laboratory of Embrapa Mandioca e Fruticultura (Table 2).

Table 2 Cassava root rot pathogens species used and GenBank accession number for the ITS rDNA region.

Voucher	Species/complex	GenBank accession number
CBPPR0006	<i>Fusarium oxysporum</i>	KT211499
CBPPR0031	<i>Fusarium equiseti</i>	KT211523
CBPPR0048	<i>Fusarium verticillioides</i>	KT211540
CBPPR0029	<i>Fusarium solani</i>	KT211521
CBPPR0012	<i>Fusarium lateritium</i>	KT211505
CBPPR0008	<i>F. chlamydosporum</i>	KT211501
CBPPR1002	<i>Lasiodiplodia theobromae</i>	KT211551
CBPPR1011	<i>Neoscytalidium dimidiatum</i>	KT211560

The isolates were transferred to Potato-Dextrose-Agar (PDA) culture medium in Petri dishes and kept in BOD-type greenhouses at a temperature of $25\pm 2^{\circ}\text{C}$ and a 12-hour photoperiod for seven days. Then, spore suspensions were performed by adding 10 mL of sterilized distilled water to the culture medium with colonies of specific isolates, using a soft toothbrush to release conidia, and the suspension was obtained by double filtration. gauze layer.

To prepare the source of inoculum, 500 g of hydrated rice were used, placed in transparent plastic bags and autoclaved at 120°C for 25 minutes and cooled to room temperature, then 20 mL of the spore suspension of each isolate was added., separately, for each 500 g of substrate. To promote the uniform growth of fungal propagules, this material was incubated in BOD, at a temperature of $25\pm 2^{\circ}\text{C}$, photoperiod of 12 hours, for 15 days, being homogenized every three days.

For soil infestation, the dry and black rot inoculum sources were mixed proportionally, and then 200g were added to each pot containing 6 L of area soil from cassava root rot, followed by homogenization of the mixture, obtaining if the final density is $5.5\times 10^5 \text{ CFU g}^{-1}$ per gram of soil.

2.2 Cassava cultivation and cover crops

In the 1st and 3rd cycles, Covering crops such as crotalaria, black oats, jack beans, maize, peanuts and cowpea were cultivated, in addition to cassava ('BRS Kiriris' and 'BRS Formosa'), interposed with fallow soil. And in the 2nd and 4th cycle, only the cassava varieties were planted, interspersed with fallow treatments, as described in 'Table 1'.

The planting of the 1st cycle with the cover crops was carried out 15 days after the soil infestation. To sow the Covering crops, 5 seeds were distributed per pot and after 15 days of planting thinning was performed, leaving 3 plants per pot. In the first and third cultivations, when the cover crops reached the stage of full flowering (>50% plants in bloom), the green manures were cut and incorporated into the soil, leaving a minimum interval of 15 days between incorporation and the new cultivation cycle aiming at the partial decomposition of cultural remains. Before the beginning of each cultivation cycle, simple soil

samples were taken at a depth of 0-10 cm from each treatment, and composite soil samples were obtained for chemical and microbiological analysis.

In all crops, before planting, the seeds of the cassava varieties 'BRS Kiriris', considered resistant to root rot (Fukuda 2002), and 'BRS Formosa', considered susceptible to root rot, were submitted to treatment with a solution of Benzalkonium Chloride (0.5mL.L^{-1} of water) for the desinfestation of the propagation material, for 3 minutes, washed in tap water and placed to dry for 12 hours at room temperature. Subsequently, two cuttings were planted per pot, according to the treatment. During the cultivation of the cassava varieties, evaluations were carried out such as germination and disease severity, and at the end of the cultivation, agronomic and disease parameters were evaluated.

2.3 Germination, agronomic and disease parameters

Germination was evaluated at 15 and 30 days after planting (DAP), during these cycles, the disease parameters were evaluated: (i) percentage of survival; (ii) severity of aerial part wilt: it was carried out at intervals of 7 days, after the first symptoms, until the final evaluation of the cultivation cycle, totaling 8 readings, through scales of notes ranging from 0 to 4, where: 0= no symptoms, 1= yellowing and/or wilting of leaves in the middle third, 2= yellowing and/or wilting of leaves in the lower and middle thirds, 3= yellowing and/or wilting of leaves on the entire plant, 4= complete defoliation and/or death.

(iii) Internal and external colonization of the cuttings, qualitatively evaluated by the presence or absence of signs and structures of pathogens, as described by Santiago et al. (2018) being the internal colonization of cuttings estimated based on the rate scale: 0=not colonized, 1=colonization $<1/3$ of the internal area of the cuttings; 2 = colonization $\geq 1/3$ and $< 2/3$ of the internal area of the stem; 3 = $\geq 2/3$ of the internal area of the stem.

The evaluations of agronomic parameters followed the methodology described by Fukuda and Guevara (1998).

- a) Number of plant stems;
- b) Plant height: measurement from the ground to the top of the plants, expressed in centimeters;
- c) Plant vigor using the following scale: 1 (little), 2 (intermediate), 3 (vigorous);
- d) Fresh weight of aerial part and roots, obtained in grams, with the aid of an analytical balance;
- e) Dry weight of aerial part and roots in grams. (For drying, the material was placed in an oven at 55°C for a period of 7 days, until it reached constant weight).

The data obtained from arbitrary rates scales (severity of wilting and severity of internal colonization) were converted into Disease Index (%), proposed by *McKinney* (1923), according to the formula:

$$DI(\%) = \left[\frac{\Sigma(\text{scale degree} * \text{frequency})}{\text{total number of units} * \text{maximum degree of scale}} \right] * 100.$$

2.4 Statistical analysis

In order to verify the relationships between the disease parameters, the averages were grouped using the Scott-Knott test ($p < 0.05$), to the evaluated parameters, the Pearson correlation test was performed, with the aid of the “GGally” package; cluster analysis using the “cluster”, “fpc” and “mclust” packages; principal component analysis (PCA), using the “factoextra”, “devtools” and “FactoMineR” packages; and the comparative distribution (boxplot), through “ggpubr”. All packages are implemented in R software version 3.6.3 (R CORE DEVELOPMENT TEAM, 2020).

2.5 Sum of ranks

To select the treatments with the best performance in soil infested by root rot, the Mulamba and Mock index (1978) was used, which initially hierarchizes the treatments for each variable, by attributing higher absolute values to those with the best performance. Then, the values attributed to each variable are added, obtaining the sum of the ranks that represents the classification of the treatments. Different weights were provided for the evaluated variables, according to the agronomic interest associated with resistance, where the sum of the ranks was obtained as: $SM = (N.Stems * 1.5) + (Height * 1.5) + (Vigor * 1.5) + (FW.AP * 1.5) + (DW.AP * 1.5) + (FW.Root * 3) + (DW.Root * 3) + (E.INC * 4) + (I.INC * 4) + (I.COL * 4) + (RES * 4) + (Sprouting * 4) + (Survival * 4)$, where N.Stems is the number of stems, Height is the height of the plants (cm), Vigor is the vigor of the plants (%), Sprouting is germination (%), Survival is survival (%), DW.AP is shoot dry weight (g), FW.AP is shoot fresh weight (g), DW.Root is the root dry weight (g), FW.Root is the root fresh weight (g), I.INC is the Internal Incidence Frequency of the stem (%), E.INC – External Incidence Frequency of the stem (%), I.COL – Internal seedling colonization (%), Res is the resistance (%), calculated based on the disease index (DI).

3. Results

Based on the average of the disease parameters in the cropping cycles grouped by the Scott-Knott test ($p < 0.05$), it was observed that in the 2nd cropping cycle there was only a significant difference for the

germination parameter, in which the bean cover crops jack bean presented inferior results to the other treatments (Fig 1). However, in the 4th cultivation cycle there was a significant difference for the parameters fresh weight of the shoot (FW. AP), dry weight of the shoot (DW. AP), fresh weight of the root (FW. Root), dry weight of the root (DW. Root) and vigor. In which the Covering crops peanut, jack bean and cowpea obtained better results regarding these parameters (Fig 2). Considering the DI (%) for all cycles, there was no significant difference (Fig 1 and Fig 2).

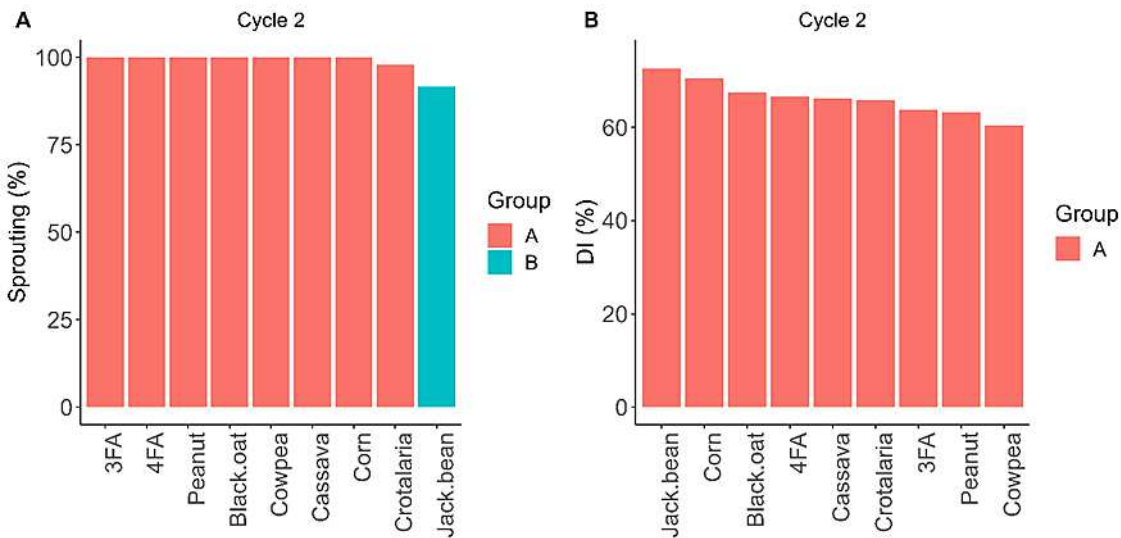


Fig. 1 Mean sprouting (%) values and DI - disease index (%), in the 2nd cassava cultivation cycle for the treatments ‘Peanut’, ‘Jack bean’, ‘Cowpea’, ‘Black oat’, ‘Crotalaria’, ‘Corn’, ‘Cassava’, and fallow, 3FA (Fallow-CA-CA-CA), 4FA (Fallow-CA-Fallow-CA). Bars with same color and capital letter belongs to the same group by the Scott-Knott test ($p < 0.05$).

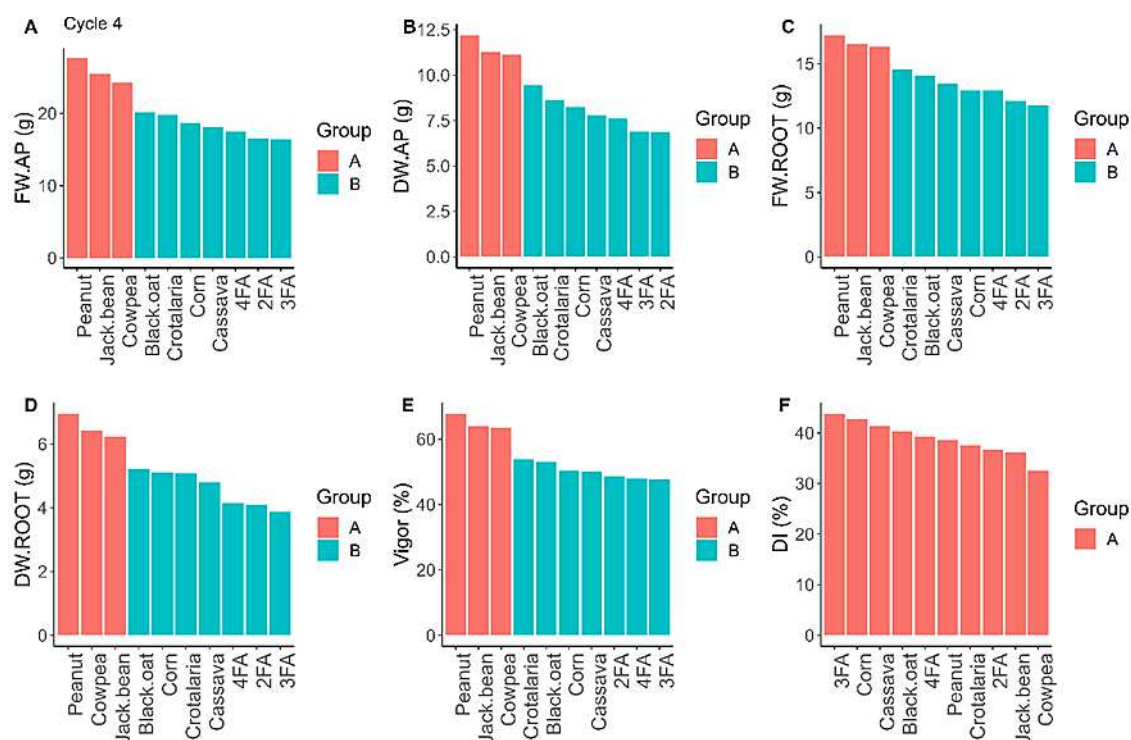


Fig. 2 Mean values of dry weight of shoot (DW.AP), Shoot fresh weight (FW.AP), root dry weight (DW. Root), Root fresh weight (FW. Root), Vigor (%): and disease index (DI) for the treatments ‘Peanut’, ‘Jack bean’, ‘Cowpea’, ‘Black oat’, ‘Crotalaria’, ‘Corn’, ‘Cassava’, and fallow, 2FA (Fallow- Fallow-CA-CA), 3FA (Fallow-CA-CA-CA), 4FA (Fallow-CA-Fallow-CA). In the 4th cycle of cassava cultivation. Bars with same color and capital letter belongs to the same group by the Scott-Knott test ($p < 0.05$)

Considering the 2nd cultivation cycle as shown in fig 3, the variable stem number showed a moderate correlation with plant height (52%), vigor (66%), FW.AP and DW.AP (41%), FW. Root (47%), DW. Root (43 %). And a strong correlation with plant survival (83%), and weak correlation with germination (28%). It showed a low negative association with the external incidence variable (33%), a moderate negative correlation with the colonization index (75%), and a strong negative correlation with the disease index (75%). Thus, there is the possibility that plants with a greater number of stems are those with a higher survival rate and, consequently, higher values for height, vigor, FW.AP, DW.AP, FW. Root and DW. Root. However, the external incidence and severity of the disease influence the amount of stem number.

Plant height showed a moderate positive association with survival (69%), FW.AP (68%), DW.AP (66%), DW. Root (68%), and a strong correlation with FW. Root (70%), and vigor (87%)., however,

showed weak positive correlation with germination (26%), and weak negative correlation with external incidence (30%) and moderate negative correlation with colonization index (41%) and DI (68%). Considering this variable, taller plants may be associated with characteristics such as better survival, FW.AP, DW.AP, DW. Root. Furthermore, observing the FW. Root variable may indicate a large influence on plant height. Despite a weak correlation, the internal incidence in the maniva interferes with height, as well as with the result of the colonization index and DI (%).

The vigor variable showed a strong positive correlation with the survival variables (77%), FW.AP (77%), DW.AP (74%), FW. Root (78%), DW. Root (77%), and a weak negative correlation with the external incidence (30%), a moderate negative correlation with colonization index (53%), and a strong negative correlation with DI (79%). By this variable, the most vigorous plants had the highest number of surviving plants, FW.AP, DW.AP, FW. Root, DW. Root. However, the variables external incidence, colonization index and DI (%) decrease plant vigor.

While germination obtained a strong positive correlation with the internal incidence of pathogens in cuttings (83%), and a weak positive correlation with the variables survival (32%), FW. Root (28%), and a weak negative correlation with DI (25%). In this sense, the plants even with internal incidence of pathogens in the stems were able to germinate, and that the variables survival and FW. Root somehow influence the germination and that the disease index reduces the germination of the plants.

Plant survival was moderately and positively correlated with the variables FW.AP (40%), DW.AP (38%), FW. Root (46%), and DW. Root (43%). And it showed a weak negative correlation with the external incidence (39%), a moderate negative correlation with the colonization index (45%), and a very strong negative correlation with the DI (90%). The plants that survived showed good characteristics regarding FW.AP, DW.AP, FW. ROOT, DW. ROOT, and regarding variables such as external incidence, colonization index and disease index, which generate a reduction in plant survival.

While FW.AP demonstrated a strong positive association with DW.AP (98%), FW. Root and DW. Root (95%). However, it showed a weak negative correlation with the colonization index (37%), and a moderate negative correlation with DI (47%). Thus, disease-related variables reduce the FW.AP.

DW.AP showed a very strong positive correlation with FW. Root (95%), DW. Root (96%) and a weak negative correlation with colonization index (39%) and a moderate negative correlation with DI (46%), it is noted, the disease variables influencing DW.AP.

The FW. Root variable showed a strong positive association with the DW. Root (97%), and a weak negative correlation with the colonization index (38%), and a moderate negative correlation with the DI (51%). While the DW. Root shows a weak negative correlation with the colonization index (38%) and a moderate negative correlation with DI (52%).

Regarding the external incidence, there are positive correlations with the colonization index variables (66%), moderate correlation and with DI (33%) weak correlation. While the internal incidence shows a weak positive correlation with the colonization index (36%). The colonization index demonstrates a weak positive correlation with DI (39%). The variables for disease are related, they may be associated, causing a reduction in the agronomic characteristics of the plants.

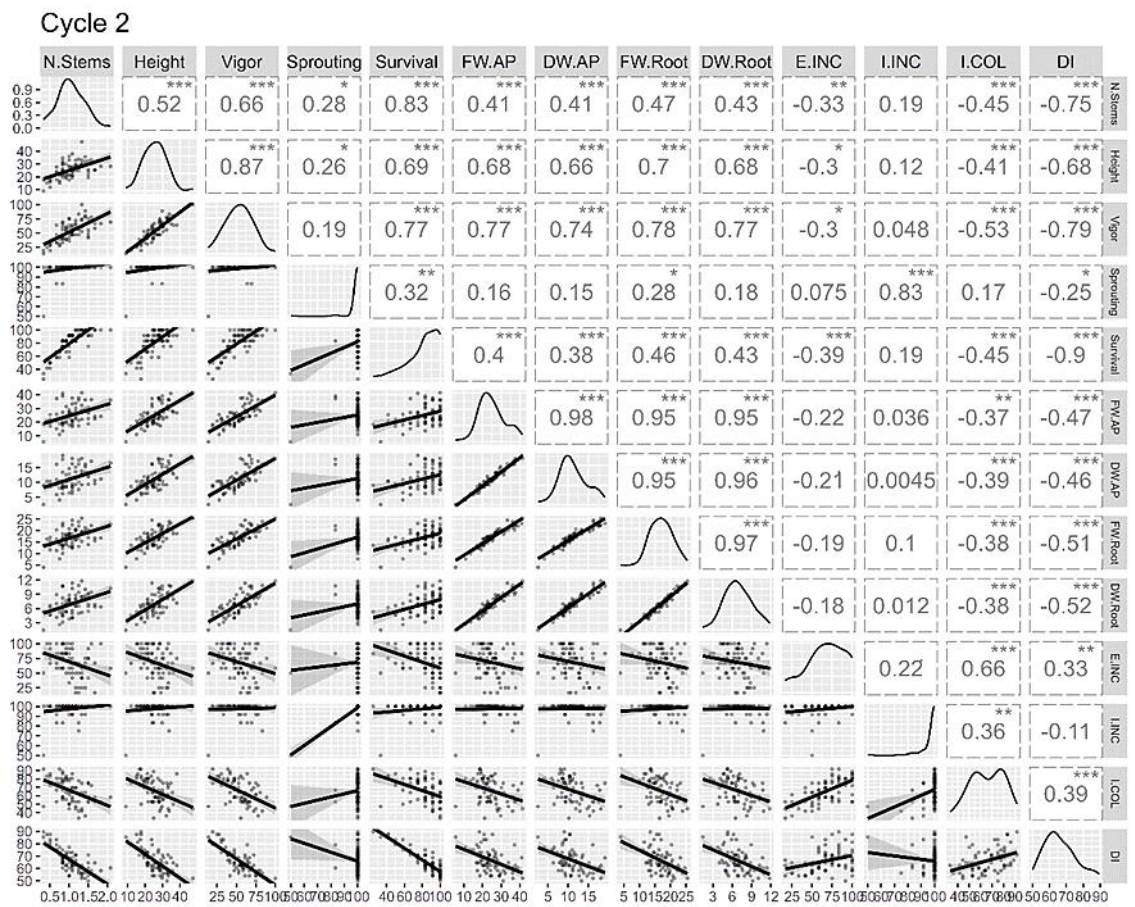


Fig. 3 Pearson correlation between agronomic and disease parameters, referring to the 2nd cultivation cycle of 2 cassava genotypes cultivated in soils infested by dry and black rot fungi of cassava roots, submitted to a succession of cover crops and fallow lands. N.Stems: Stem number. Height: Height (cm). Vigor: Vigor (%). Sprouting: Germination (%). Survival: Survival (%). DW.AP: Dry weight of shoot (g), FW.AP: Shoot fresh weight (g), DW. Root: root dry weight (g), FW. Root: Root fresh weight (g). I.INC: Internal

Incidence Frequency (%). E.INC: External Incidence Frequency (%). I.COL: Maniva Internal Colonization (%). DI: Final Disease Index (%). Significance level α : * =0.001; ** = 0.01;***=0.1.

Evaluating the variables in the 4th cultivation cycle, as shown in fig 4, it is observed that the number of stems in the plant has a weak positive correlation with height (28%), vigor (38%), sprouting (24%), FW. Root (32%), DW. Root (25%), and a moderate positive correlation with the plant survival variable (62%). In addition, it obtained a moderate negative correlation with the colonization index (45%) and a weak negative correlation with DI (36%). The plants that have more number of stems, even showing a weak correlation with height, vigor, germination, FW. Root, DW. Root, and moderate correlation with survival, these characteristics are the ones that possibly influence plants with a greater number of stems. However, the colonization index and DI (%) can reduce the amount of stem number.

Regarding plant height, there was a strong positive correlation with the variable vigor (73%), and a weak positive correlation with plant survival (36%), and a moderate positive correlation with the variables FW.AP (69%), DW.AP (61 %), FW. Root (65%), DW. Root (58%). In addition, it showed a moderate negative correlation with the colonization index (46%) and a weak negative correlation with the external incidence (27%) and DI (32%). While this variable taller plants may show better vigor, survival and FW.AP, DW.AP, FW. Root, DW. Root. However, the colonization index, external incidence and DI (%) results affect plant height.

As for vigor, it showed a high positive association with FW.AP (83%), DW.AP (79%), FW. Root (81%), DW. Root (76%). Low positive association with the survival variable (31%). However, it showed a moderate negative correlation with the colonization index (40%) and a weak negative correlation with DI (39%). More vigorous plants will have plants with more FW.AP, DW.AP, FW. Root, DW. Root. However, variables related to the disease reduce the vigor of the plant.

Plant germination only showed significant values for survival (51%), moderate positive correlation and for DI (%) a weak negative correlation of 36%. While plant survival showed a weak positive correlation with FW. Root (25%), and external incidence (25%), it showed a weak negative correlation with colonization index (29%) and a moderate negative correlation with DI (65%). The survival variable is influenced by FW. Root, so plants with better FW. Root tend to be able to survive, and even with external incidence the survival of plants, but the variables colonization index and disease index demonstrate that the severity of the disease can reduce the survival of the plants.

As for FW.AP, it showed a strong positive association with DW.AP (96%), FW. Root (88%), and DW. Root (87%). And weak negative correlations with external incidence (29%), internal incidence (24%) and colonization index (34%). As for the DW.AP, it showed a strong correlation with the FW.Root (85%), DW. Root (88%) and a low negative association with the variables external incidence (35%), internal incidence and colonization index (29%). Regarding the FW. Root, it showed a high positive association with the DW. Root (88%), and weak negative correlations with the colonization index (28%) and ID (39%). As for the DW. Root, it showed a weak negative correlation with the colonization index (23%) and DI (28%).

Assessing the external incidence, it showed a high positive association with the internal incidence (86%) and a moderate positive association with the colonization index (45%). Thus, the Internal incidence showed a moderate positive association with the colonization index (43%), while the colonization index correlated weakly and positively with DI (28%).

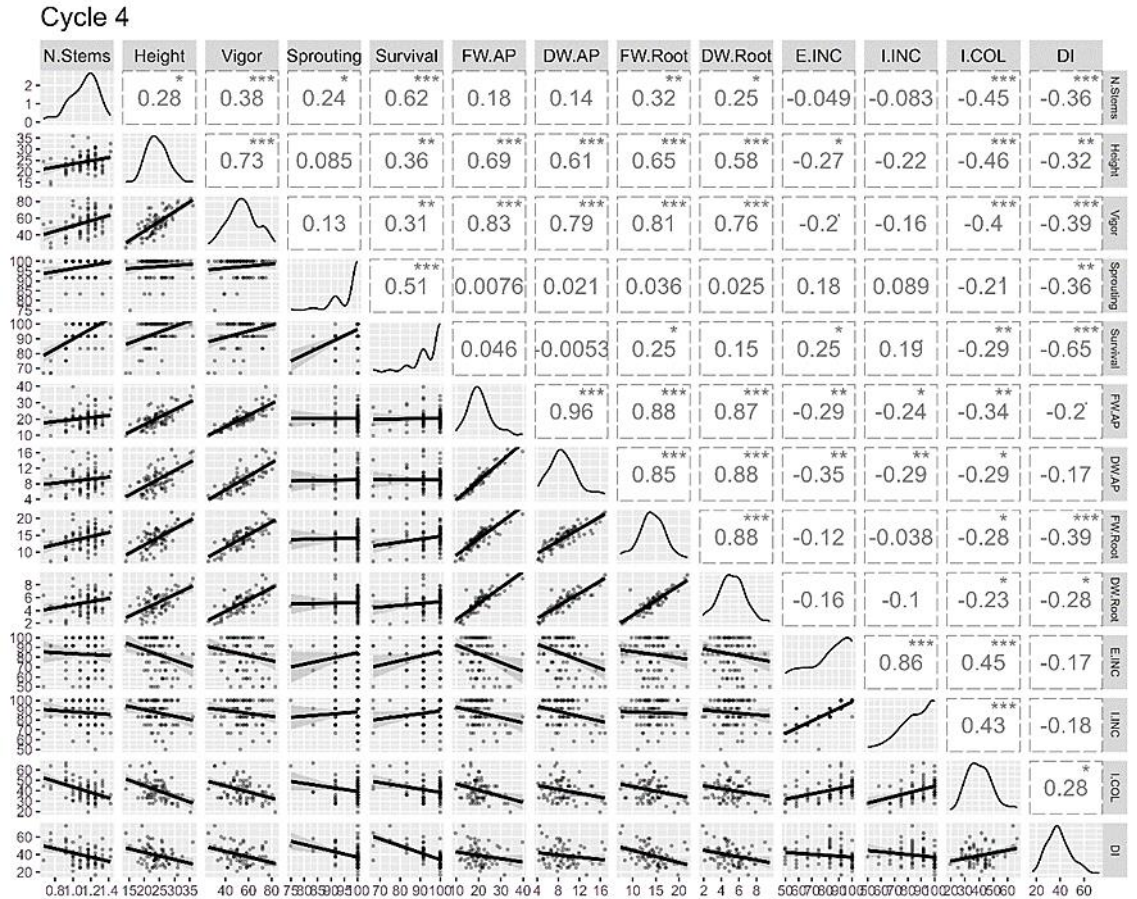


Fig. 4 Pearson's correlation between agronomic and disease parameters, referring to the 4th crop cycle of 2 cassava genotypes grown in soils infested by dry and black rot fungi of cassava roots, submitted to a succession of cover crops crops and fallow lands. N.Stems: Stem number. Height: Height (cm). Vigor:

Vigor (%). Sprouting: Germination (%). Survival: Survival (%). DW.AP: Dry weight of shoot (g), FW.AP: Shoot fresh weight (g), DW. Root: root dry weight (g), FW. Root: Root fresh weight (g). I.INC: Internal Incidence Frequency (%). E.INC: External Incidence Frequency (%). I.COL: Maniva Internal Colonization (%). DI: Final Disease Index (%). Significance level α : * =0.001; ** = 0.01;***=0.1.

Comparing the 2nd and 4th cycle of cultivation in fig 3 and 4, there was a reduction in the correlation between the disease index and the agronomic parameters. For the correlations became weak negative. In addition, due to the succession of crops, some variables that previously had a strong to moderate correlation became weak and some did not change the association with each variable. The variables that continued to have a strong correlation both in the 2nd and 4th cycle of cultivation were in relation to plant height, which was strongly correlated with vigor, FW.AP, DW.AP, FW. Root, DW. Root. Another variable, such as vigor, was considered important for both cycles: FW.AP, DW.AP, FW. Root, DW. Root. In addition, fresh and dry weights for shoots and roots in all cultivation cycles were strongly correlated.

3.1 Principal Components Analysis (PCA)

In the analysis of main components of cover crops and fallow land in the 2nd cassava crop cycle related to the 'BRS Formosa' and 'BRS Kiriris' varieties, allowed the formation of 3 groups (Fig 5A), thus, group 1 (Black oats, 4 fallow), group 2 (jack beans and maize), group 3 (cowpea beans, cassava, crotalaria, peanuts, 3 fallow). Dim 1 accounted for 39.7% and Dim 2 for 35.3% of the total variation of these treatments. Note that the height (Height), survival (survival), germination (sprouting), stem number (N. Stems) and vigor are inversely proportional to the variable disease index (DI). There are high correlations between the FW variables. AP, DW.AP, FW. Root, DW. Root, also between colonization index and external incidence.

Analyzing the main components, there were lower estimates for the variables Height, Survival, I. INC, vigor and N. Stems and higher DI, considering the vegetable cover, jack bean and maize, belonging to group 2. The treatments that presented the highest estimates for vigor, N. Stems, Survival, height, sprouting, I.INC and lowest DI (%) were the cultures belonging to group 3, but those with the highest estimates for the FW variables. AP, DW.AP, FW. Root, DW. Root, E. INC, I. COL, were the cultures belonging to group 2, but they presented high DI (Fig 5A).

As for the fourth crop cycle (Cycle 4), evaluating the cover crops relating them to cassava varieties and the agronomic and disease variables, the main component 1 and 2 was obtained, explaining

49.2% and 22.5%, respectively their variability, thus allowing to explain 71.7% of the total variation (Fig 5B). It is observed that N. Stems and survival are inversely proportional to I. INC, E. INC, I. COL, DI, in addition to the variables N. Stems and survival such as I.INC, E. INC, in addition to the index of colonization and disease index, as well as the variables FW.AP, DW.AP, DW. Root, FW. Root, vigor, show high correlations with each other.

According to the main components, the cowpea crop, belonging to group 5, showed a low estimate for the variables I. INC, E. INC, I. COL, DI, and a high estimate for the variables Height, N. stems, Survival. On the other hand, group 2 (cassava, corn, crotalaria, 4 fallow) presented high estimates regarding I. INC, E. INC, I. COL, DI, while the cultures of group 4 (jack beans and peanuts) presented high values referring to the variables FW.AP, DW.AP, DW. Root, FW. Root, vigor, Sprouting (Fig 5B).

Comparing the cover crops, it can be seen that both in the 2nd and 4th cultivation cycles, the cowpea crop presented high estimates for height, N. stems, survival and low values for DI. While jack beans showed high values for the variables FW.AP, DW.AP, DW. Root, FW. Root. Already the peanut culture for characteristic vigor and height. While, corn showed high values for DI, E. INC, I. COL and cassava culture high values of I. INC in the 2nd cycle and I. COL, DI in the 4th cycle of cultivation.

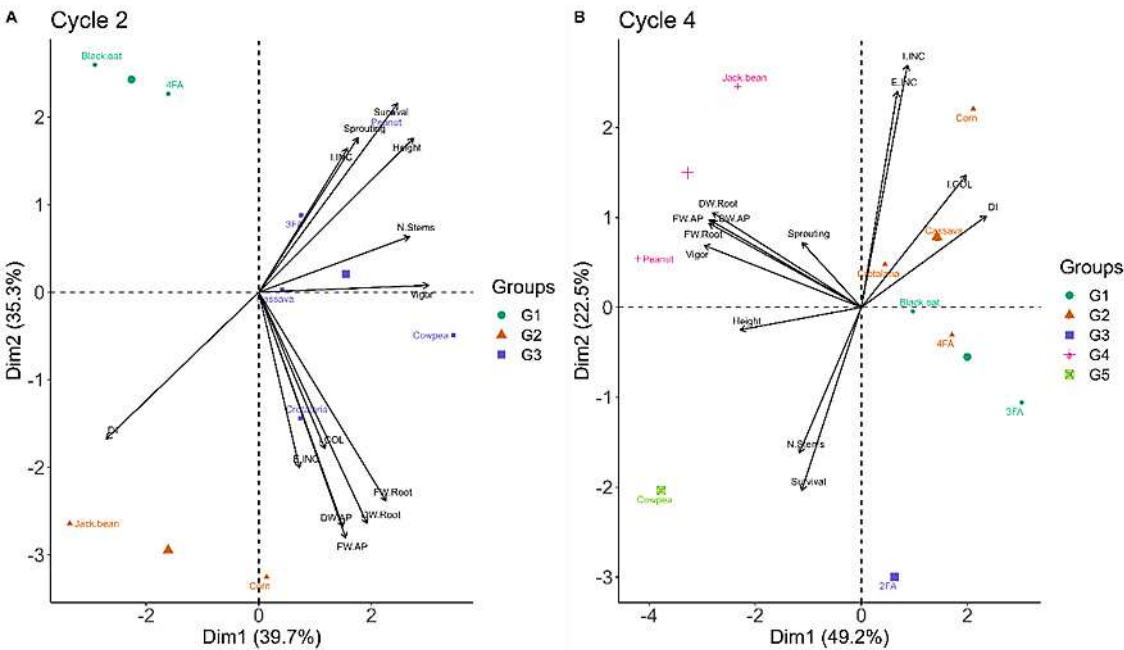


Fig. 5 Projection diagram of vectors of agronomic attributes and diseases related to groups of vegetation cover and fallow in the 2nd cropping cycle (A) and in the 4th cropping cycle (B). The variables presented are: N. Stems (Number of stems), Height (height), vigor, Sprouting (germination), Survival (survival), DW.AP (Dry weight of shoot), FW.AP (Shoot fresh weight), DW. Root (root dry weight), FW. Root (Root

fresh weight), I. INC (Internal incidence frequency of the stem), E. INC (External incidence frequency of the stem), I. COL (Internal seedling colonization), DI (disease index). The crops mentioned in the groups are: 'Peanut', 'Jack bean', 'Cowpea', 'Black oat', 'Crotalaria', 'Corn', 'Cassava', and fallow, 2FA (Fallow-Fallow-CA-CA), 3FA (Fallow-CA-CA-CA), 4FA (Fallow-CA-Fallow-CA).

When comparing the 2nd cultivation cycle and the 4th cultivation cycle, the possible cover crops to be used in succession of crops to reduce cassava root rot and bring better agronomic characteristics based on the results presented are cowpea, jack bean and peanuts.

3.2 Box Plot Result

The parameters chosen for the distribution analysis (Boxplot) were based on the PCA analysis, selecting the variables that most contributed to the variability of the data, both in the 2nd cycle and in the 4th cycle of cassava cultivation. Observing figure 6, for the final disease index (DI) variable, group 3 formed by crotalaria, cassava, 3 fallow, peanuts, cowpea, showed the greatest reductions in the DI, and as for the comparison between groups, the comparison between G3 and G2. As for the survival parameter, the comparison between G3 and G2 was significant. Since G3 had more surviving plants. Regarding the height parameter, the comparison between G3 and G2 was significant, but visually, G3 had taller plants. As for the vigor parameter, the comparison between G1 and G3 were significant, but G3 visually obtained plants with more vigor (Fig 6).

Considering the FW.AP and DW.AP parameters, the comparisons between the groups were not significant, however G2 visually stood out in these parameters (Fig 6). As for FW. ROOT, the comparison between groups G3 and G1 were significant, but visually, G3 showed plants with more FW.ROOT. As for the DW. ROOT, the comparison between G3 and G1, G1 and G2 were significant.

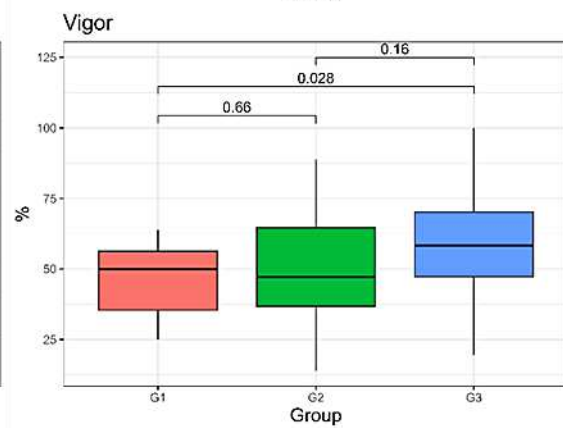
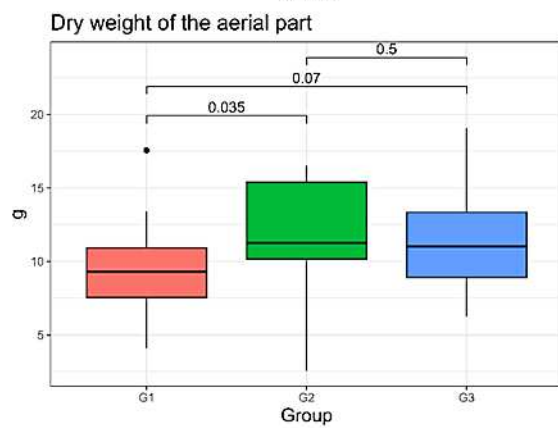
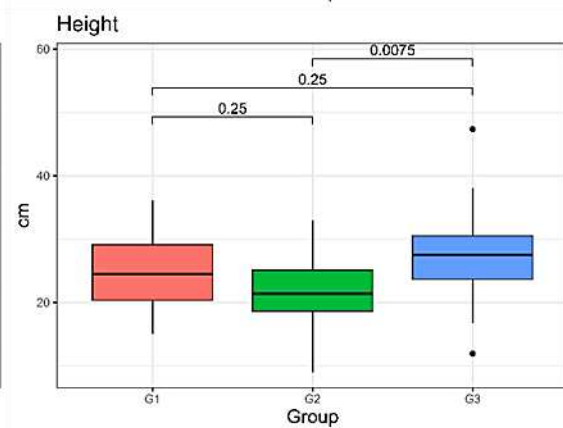
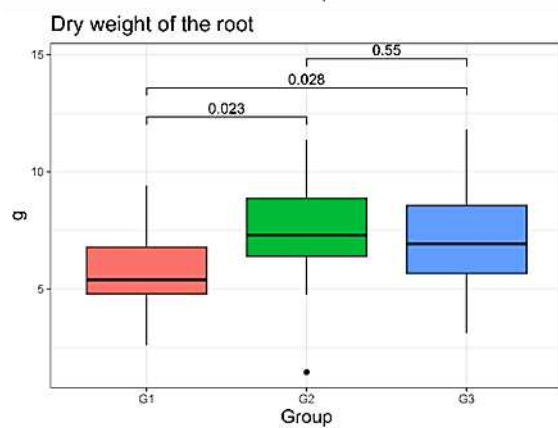
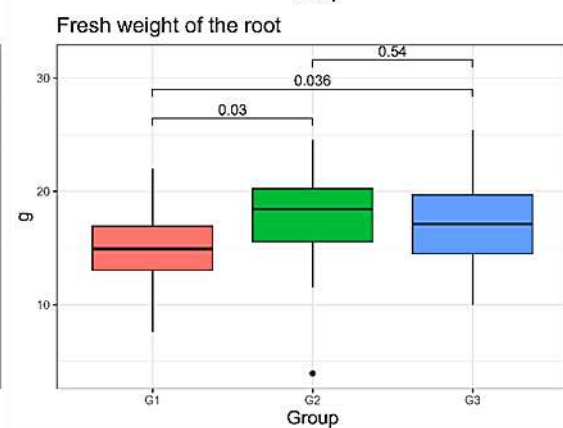
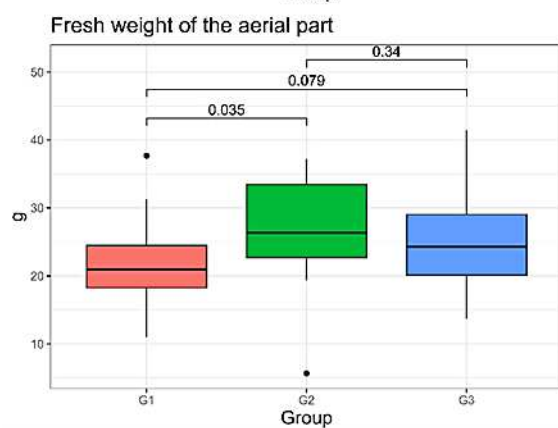
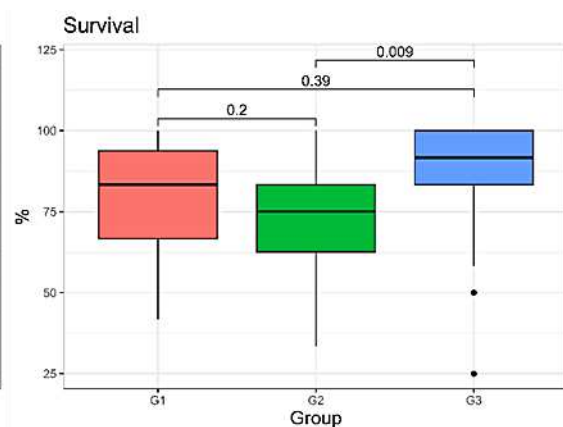
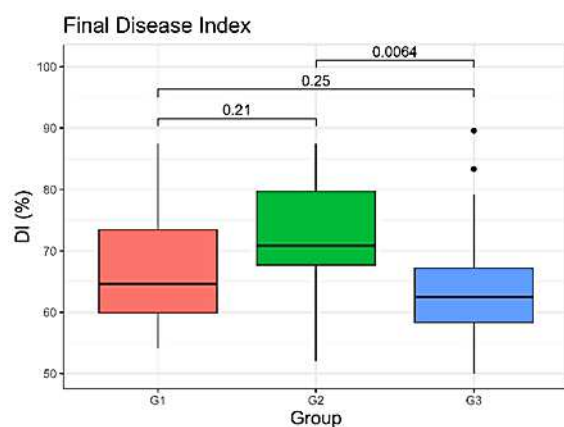


Fig. 6 Boxplot of the groups formed based on the variables root dry weight (g), shoot dry weight (g), height (cm) and vigor (%), referring to the 2nd cultivation cycle of the groups: G1 (black oats, 4 fallow), G2 (Jack bean, corn) and G3 (crotalaria, cassava, 3 fallow, peanut, cowpea).

In the 4th cassava cultivation cycle, G5 represented the crops with the lowest DI%, and the lowest internal incidence. In the comparisons between groups G1 and G5, G2 and G5 were significant, and visually G5 represented the lowest DI%. As for the internal incidence parameter, comparisons between groups were not significant. Evaluating the survival parameter, there was a significant difference between G1 and G5, but G5 visually showed more surviving plants. Considering the vigor parameter, comparisons between groups G3 and G4, G3 and G5, G1 and G4, G1 and G5, G2 and G4, G2 and G5 were significant. Since the groups G4 and G5, respectively, were the ones with the most vigorous plants (Fig 7).

As for the FW.AP, comparisons between groups G3 and G2, G3 and G4, G3 and G5, G1 and G4, G2 and G4. They were significant. Since G5, followed by G4, were the ones that presented the most FW.AP. Considering the DW.AP, the comparison between groups G3 and G4, G3 and G5, G1 and G4, G1 and G5, G2 and G4, G2 and G5 were significant. Observing Figure 5, G5 and G4, respectively, were the groups with the highest DW.AP (Fig 7).

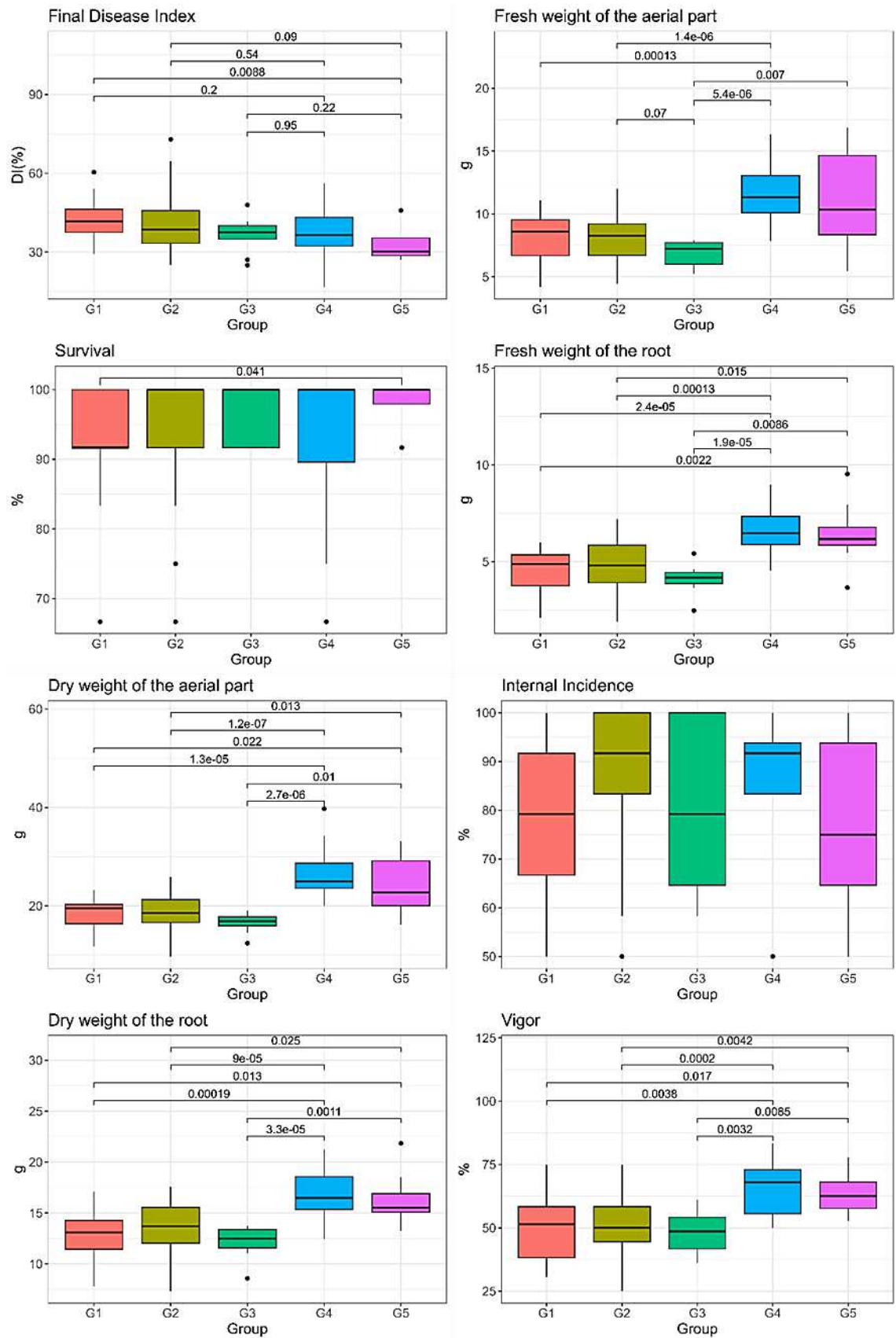
Analyzing the FW. ROOT, the comparisons between the following groups: G3 and G4, G3 and G5, G1 and G4, G1 and G5, G2 and G4, G2 and G5 were significant, and visually by the graph the groups G4, G5 were the ones that most represented the amount of FW.ROOT. And as for the DW. ROOT, the comparisons between the groups G3 and G4, G3 and G5, G1 and G4, G1 and G5, G2 and G4, G2 and G5 were significant. Visually evaluating the graph, groups G4, G5 respectively, presented the highest DW. ROOT (Fig 7).

Based on the sum of the treatment rankings for each of the parameters evaluated in the 2nd cassava cultivation cycle, the best treatment in terms of height (29.17%), survival (91.67%) I.COL (69.10%) was peanut, while in N.Stems (1.19%) it was peanut and cowpea. However, cowpea also presented favorable results for vigor (62.50%) and DI reduction (60.42%), while crotalaria stood out for the FW parameters. AP (27.89%), DW.AP (13.00%), and reduction in E.INC (77.68%), and corn showed good results for FW.Root (18.70%), DW.Root (8.00%). The Jack bean treatment presented the worst results in terms of lower germination rate (91.67%), height (20.34%), DI (72.66%) and survival (70.83%), as shown in table 3.

In the 4th cassava cultivation cycle, the best treatments were cowpea, peanut, 2FA, and jack bean, considering the parameters vigor (67.70%), height (27.75%), FW.AP (27.66%), DW.AP(12.18%),

420 FW.Root (17.20%), DW.Root (6.94%) the best treatment was peanut, however the cowpea treatment is also
421 one of the best considering N.Stems (1.24%), survival (97.92) and reduction in I.INC (81.25%) and DI
422 (32.55%), while the jack bean and 2FA treatments stood out in terms of germination (98.96%) and reduction
423 of I.COL (35.76%), respectively (table 4). Comparing the cultivation cycles, cowpea showed resistance

424 based on the reduction of DI (%) in both the 2nd cycle (72.66%) and the 4th cultivation cycle (32.55%).



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426 **Fig. 7** Boxplot of the groups formed based on the variables disease index (%), internal incidence (%),
427 survival (%), vigor (%), fresh weight of the shoot (g), dry weight of the shoot (g), root fresh weight (g),

- 428 root dry weight (g). Referring to the 4th crop cycle of the groups: G1 (3 fallow, black oat), G2 (crotalaria,
429 cassava, corn, 4 fallow) and G3 (2 fallow), G4 (peanut, jack bean), G5 (cowpea).

430 **Table 3.** Means of the parameters of the 2nd cassava crop: Vigor (vigor), Height (height), N. Stems (Number of stems), Sprouting (germination), Survival (survival), FW.AP (

431 Shoot fresh weight), DW.AP (Dry weight of shoot), FW. Root (Root fresh weight), DW. Root (root dry weight), E. INC (External incidence frequency of the stem), I. INC

432 (Internal incidence frequency of the stem), I. COL (Internal seedling colonization), DI (disease index) to evaluate best treatment performance.

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Cycle 2		Parameters												
Sum RK (MM)	Treatments	Vigor	Height	N.Stems	Sprouting	Survival	FW.AP	DW.AP	FW.Root	DW.Root	E.INC	I.INC	I.COL	DI
1	CROTALARIA	54.51	25.92	1.04	97.92	78.13	27.89	13.00	18.15	7.79	58.33	96.88	63.90	65.89
2	PEANUT	62.15	29.17	1.19	100.00	91.67	24.68	10.83	16.85	6.80	66.67	100.00	63.54	63.28
3	COWPEA	62.50	27.74	1.19	100.00	89.58	26.27	11.66	18.31	7.52	77.08	96.88	68.06	60.42
4	3FA	55.90	27.90	1.03	100.00	84.38	24.71	11.17	16.53	6.65	68.75	97.92	65.98	63.80
5	CASSAVA	52.08	25.26	1.06	100.00	82.29	24.35	11.26	17.15	7.10	67.71	98.96	67.71	66.15
6	4FA	46.18	24.81	1.02	100.00	82.29	22.81	10.21	15.27	5.91	60.42	100.00	64.93	66.67
7	CORN	50.35	23.48	0.96	100.00	72.92	27.77	12.34	18.70	8.00	77.08	98.96	69.10	70.57
8	BLACK.OAT	47.22	24.48	0.95	100.00	79.17	21.29	9.17	14.93	5.67	62.50	96.88	63.54	67.45
9	JACK.BEAN	49.30	20.34	1.01	91.67	70.83	25.59	11.39	16.31	6.77	75.00	91.67	64.93	72.66
	Minimum	46.18	20.34	0.95	91.67	70.83	21.29	9.17	14.93	5.67	58.33	91.67	63.54	60.42
	Maximum	62.50	29.17	1.19	100.00	91.67	27.89	13.00	18.70	8.00	77.08	100.00	69.10	72.66
	Mean	53.35	25.45	1.05	98.84	81.25	25.04	11.22	16.91	6.91	68.17	97.57	65.74	66.32

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 442 **Table 4.** Means of the parameters of the 4nd cassava crop: Vigor (vigor), Height (height), N. Stems (Number of stems), Sprouting (germination), Survival (survival), FW.AP (Shoot fresh weight), DW.AP (Dry weight of shoot), FW. Root (Root fresh weight), DW. Root (root dry weight), E. INC (External incidence frequency of the stem), I. INC (Internal incidence frequency of the stem), I. COL (Internal seedling colonization), DI (disease index) to evaluate best treatment performance.

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Cycle 4		Parameters												
Sum RK (MM)	Treatments	Vigor	Height	N.Stems	Sprouting	Survival	FW.AP	DW.AP	FW.Root	DW.Root	E.INC	I.INC	I.COL	DI
1	COWPEA	63.54	25.02	1.24	97.92	97.92	24.27	11.14	16.33	6.41	77.08	81.25	37.85	32.55
2	PEANUT	67.70	27.75	1.13	97.92	95.83	27.66	12.18	17.20	6.94	80.21	87.50	37.15	38.54
3	2FA	48.61	24.82	1.18	97.92	96.87	16.57	6.87	12.11	4.10	80.21	82.29	35.76	36.72
4	JACK.BEAN	63.88	25.68	1.05	98.96	90.63	25.46	11.28	16.51	6.24	87.50	90.62	39.23	36.20
5	CROTALARIA	53.81	24.29	1.13	96.88	93.75	19.84	8.63	14.56	5.07	86.46	87.50	44.44	37.50
6	BLACK.OAT	53.12	21.48	1.14	93.75	90.62	20.19	9.42	14.07	5.21	81.25	85.42	40.28	40.36
7	4FA	47.91	23.98	1.08	98.96	94.79	17.49	7.63	12.92	4.15	79.17	89.58	42.71	39.32
8	CASSAVA	50.00	23.67	1.09	98.96	94.79	18.16	7.79	13.44	4.79	87.50	91.67	40.97	41.41
9	CORN	50.34	22.33	1.15	97.92	92.71	18.66	8.24	12.92	5.12	94.79	95.83	43.74	42.71
10	3FA	47.56	24.09	1.06	93.75	92.71	16.46	6.89	11.76	3.87	77.08	84.38	42.36	43.75
	Minimum	47.56	21.48	1.05	93.75	90.62	16.46	6.87	11.76	3.87	77.08	81.25	35.76	32.55
	Maximum	67.70	27.75	1.24	98.96	97.92	27.66	12.18	17.20	6.94	94.79	95.83	44.44	43.75
	Mean	54.65	24.31	1.12	97.29	94.06	20.48	9.01	14.18	5.19	83.12	87.60	40.45	38.91

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4. Discussion

The effect of cultivation and incorporation of different cover crops in the soil, resulted in different results regarding the ability to suppress dry and black root rot. Many studies find the unpredictability of the effects of organic materials, plant types, or management on pathogen population and soil suppressiveness (Bonanomi et al., 2007). Therefore, some cover crops or strategies used can generate an increase or decrease in the disease, especially in specific soil diseases. In view of this, the importance of knowing the effects of different cover crops regarding the impact on the cassava crop.

Because of the cropping cycles using specific cover crops, there was a reduction in the disease and mainly benefited the agronomic characteristics of the plants. These cultivation cycles affected the growth of inoculums and disease development, since the rotation or succession of cultivations end up affecting the breakdown of pathogen development. The cultivation and incorporation of cowpea, jack bean and peanut cultures were the ones that presented the best performance in terms of suppressing the disease and providing good agronomic characteristics to the cassava varieties 'BRS Kiriris' and 'BRS Formosa'.

Thus, considering the results among the coverages to be used in succession of crops with cassava 'BRS Kiriris' and 'BRS Formosa' are the legumes represented by cowpea, jack bean and peanut, as the cowpea crop provided greater reduction of disease and good agronomic characteristics, while jack bean and peanut showed good results regarding cassava agronomic parameters.

As with other crops, these cover crops can be used in management to improve soil quality, microbial diversity and crop yields. According to Salem et al., (2020) suggested that root growth of cover crops can provide benefits to the soil, including increases in soil organic carbon content, available nutrients, as well as soil aggregation. In addition, cover crops have been found to improve nutrient cycling, soil physicochemical and biological properties, as well as soil pest management and weed suppression.

The legumes used in the work were more efficient than grasses, this may have occurred due to their rapid decomposition, which can release captured nutrients back into the system more quickly than grasses (Koudahe et al., 2022). In addition, one should consider its ability to obtain nitrogen through the symbiotic relationship with bacteria in the root nodules, fixing nitrogen from the atmosphere. Thus, the three leguminous crops were more efficient in terms of disease reduction and the performance of the agronomic characteristics of the cassava varieties.

Although crotalaria is also a legume, it did not achieve as many positive results as the others, this may have occurred because different legume species can fix different amounts of nitrogen depending

on the cumulative production of biomass (Koudahe et al., 2022). Legumes that produce more biomass will provide greater amounts of fixed nitrogen. In addition, due to the symbiotic relationship with rhizobium, legumes can take advantage of atmospheric nitrogen that bind to plant tissues. In this way, this nitrogen becomes available for the subsequent crop as the biomass of the cover crops is reduced. In addition, that goes for the cassava crop, despite being tolerant and with few nutritional requirements, it is more productive if it is nutritionally well. Moreover, legumes favor this characteristic mainly when it comes to agronomic characteristics.

Knowledge about the agricultural characteristics of the varieties is essential for combating root pathogens, as one of the main methods for managing fungal diseases is the use of resistant hosts. Expected to behave as resistant, however in the 2nd cycle of cultivation, it behaved as susceptible to root rot, showing similarities with results found by Santiago et al., (2018) who, when using soil infested with dry rot isolates, culminated in the appearance of symptoms and death of these plants, grouping it in the most susceptible varieties. This may have occurred due to 'BRS Kiriris' being susceptible to the pathogen complex and as reported by Boas et al (2016) that this variety could have greater susceptibility when root rot was caused by *Fusarium*. While the variety 'BRS Formosa' is known to be susceptible to root rot, however it behaved as moderately resistant, showing good results in agronomic parameters in the 2nd and 4th cycle of cultivation. with better vigor, height, FW.AP and DW.AP. This behavior is similar to that found by Santiago et al., (2018) who, in evaluations of different forms of root rot inoculation, belonged to the group of varieties with greater resistance to dry root rot and black root rot, but there is still no data to indicate why this behavior of 'BRS Formosa' in some studies involving cassava root rot.

The incorporation of plant cover in the soil throughout the cultivation cycles provided good agronomic characteristics for the cassava varieties; similar to what was observed by Tilston et al., (2005) where the increase in the levels of organic matter in the soil may be responsible for the better performance of the plants. For even when compounds are not directly suppressing the phytopathogens, plant growth and vigor can be stimulated or induced by increasing nutrient uptake. According to De Corato (2020) certain non-suppressive compounds can even stimulate plant growth and vigor and/or induce soil nutrient uptake by plant tissue, to promote crop productivity. As observed by the use of cover crops that did not present suppressive characteristics to the disease, but may have influenced the agronomic characteristics of the plants.

The increase in vigor of cassava varieties with the incorporation of cover crops (FC, FP, AM) may have been influenced by changes in the abiotic characteristics of the soil. As noted by Ghorbani et al (2008), Fang et al (2012), in which the role of organic changes in the soil in suppressing the disease may be associated with the reduction of aggressiveness and infection of pathogens as well as the increase in plant vigor as result of the physical and chemical improvement of the soil. Furthermore, it may have influenced the results of the 'BRS Kiriris' variety, in which, in the 4th cultivation cycle, it obtained improvements in the characteristics of vigor and reduction of the disease index, possibly due to the same effect that is attributed to compounds that through mechanisms such as improving plant nutrition and vigor can increase disease resistance (St Martin and Ramsubhag, 2015).

Cover crops and rotation can reduce or increase the severity of plant disease by a variety of mechanisms. These effects may be variable between plant species (Abawi and Widmer, 2000) and cultivar-to-cultivar (Mazzola and Gu, 2002). Observed in the 2nd and 4th cycle of cultivation where depending on the species of plant selected for incorporation into the soil, there was an increase in the severity and index of the disease and while the use of different varieties of cassava resulted in a reduction or increase in the incidence of the disease. Therefore, plant selection and handling are essential to achieve disease suppression and better plant agronomic characteristics, according to Mazzola et al., (2001) and Pérez-Piqueres et al., (2006), the selection of crops is important for the efficient management of the disease, as some crops used for this purpose can increase the severity of the disease, as occurred with the Covering crops maize, black oats, cassava, and fallow lands that were not effective in suppressing of the disease and had a higher incidence and severity of the disease.

The maize crop showed a higher rate of disease and colonization by pathogens on the cuttings, it is worth mentioning that maize cultivation is frequent in monoculture, generating an increase in the incidence of pathogen populations, and in the present study this situation was confirmed, corroborating the results found by Awoyemi et al., (2016) in which the intercropping of maize with cassava does not have management potential in the development of root rot. However, it differs from what was found by Barros et al., (2014) and De Medeiros et al., (2019) who presented it as one of the possible plant covers that can influence soil suppression when intercropped with beans and cassava; however, the planting of this plant was for a long period, unlike what happened in the present study.

When testing the treatment (MA-MA-MA-MA), there was a higher frequency of external incidence of pathogens in the propagation material. As Abawi and Widmer (2000) reported that root

diseases are prevalent when susceptible crops are used in a sequence, leading to the accumulation of high population densities of plant pathogens. Therefore, one must consider the factors that are influential in the selection of crops that affect the quality and quantity of residues incorporated or left under the soil, which are: sequence of cultivation and the periodicity of crop rotation (short cycles X long cycles) and crop management (fallow land, ploughing, etc.).

The selection of cover crops is essential for the success of integrated management for the cassava crop, so choosing the ideal crop is necessary for good agronomic performance and disease reduction. In addition to the selection of the cover crops, the quality of incorporation of the material to the soil, the period of succession of crops and the quality genetic material are important, they are factors that contributed to the suppression of the soil and consequently to the productivity of the plant.

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Author contributions

Planning and designing the research, Saulo A.S.O. and Maria C.P.S., methodology, Maria C.P.S., Saulo A.S.O., Maria S.A.S.D. and Camila S.H.; implementation and conduction of the trials, Maria C.P.S. and Cristiana B.M.V.; writing—original draft preparation, Maria C.P.S. and Saulo A.S.O.; writing—review and editing, Maria C.P.S., Maria S.A.S.D., Camila S. H., Cristiana B.M.V. and Saulo A.S.O. All authors have read and agreed to the published version of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflicts of interest The authors declare no conflict of interest

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