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Comparative Study Between ATOMES Novels, NOVOSECT SC21®, ATO BED BUG® and NEO-BOOST® as a Bio-organic Solution in Managing Tomato Open Field Plantation in Hrajel Area in Lebanon

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Abstract

The increasing use of chemical insecticides has adversely effected the environment and increased insect resistance. Biopesticides have been noticed the potential to be an excellent alternative to chemicals to reduce the negative impacts to human health and the environment. Tomato (*Lycopersicon esculentum*) is the second most important vegetable crop worldwide due to its nutritional importance. The effect of NOVOSECT SC21® (0.5 L/200 L), ATO BED BUGS® (1 L/200 L) and NEO-BOOST® (1 kg/200 L) against *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani* was studied. A complete randomized block design (CRBD) was used with three replications, three treatments and one control in Hrajel area in Lebanon in the summer of 2020. We evaluated the level of infestation and larval mortality level of *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani* infection 24, 48 and 72 hr after the application. Results showed that NOVOSECT SC21® (Mix of metabolites of the Bacillus F.D. 777) was the most efficient in decreasing the infestation and inducing larval mortality level of *T. absoluta* and *L. trifolii* and the fungal infection induced by *A. solani* with significant difference with time, followed respectively by ATO BED BUGS® and finally NEO-BOOST®.

Keywords: *Alternaria solani*, ATO BED BUGS®, *Liriomyza trifolii*, NOVOSECT SC21®, NEO-BOOST®, Tomato, *Tuta absoluta*

1. Introduction

Vegetables are one of the most important crops in agriculture and tomato (*Lycopersicon esculentum*) is considered as one of the most cultivated and consumed vegetable crops in Lebanon, with a production of 300,157 tons in 2018 and a cultivated area of 3700 ha according to IDAL (Investment Development Authority in Lebanon) (IDAL, 2020). Over the years, the increased demand for vegetables has resulted in the increase in land cultivation and the adoption of intensive farming in greenhouses. However, the increasing pressure on the soil and the excessive use of chemical fertilizers weakens the soil and disturbs its microbiological balance leading with time to weaken seedlings, which makes them susceptible to diseases and pests.

The intensive use of chemical insecticides have serious drawbacks, including reduced profits from high insecticide costs, destruction of insect pest's natural enemy populations, the build up of insecticide residues on tomato fruit. In addition, the high use of chemical insecticides can adversely affect the environment and increase the insecticide resistance to many insects, including *Tuta absoluta* and *Liriomyza trifolii*, which are among the most important tomato pests in Mount Lebanon, causing severe problems to tomato crops and significant losses in production that could reach 100% if not controlled (Devine et al., 2007; Giorgini et al., 2018; MOA, 2016). The need to adopt new technologies and good agricultural practices should be taken into consideration in order to lower the production costs, lower the insecticide residues in the product, protecting the environment, and obtaining effective insect control (IDAL, 2020). Natural products are an excellent alternative to synthetic pesticides as they help reducing their negative impacts on human health, while protecting the environment and

beneficial pest population. Natural products (biopesticides) are safe and eco-friendly and more compatible with the environmental components than synthetic pesticides (Isman, 2000; Isman & Machial, 2006). Biopesticides include substances such as plant extracts, hormones, pheromones, entomophagous control, plant derived pesticide, etc. (Koul, 2008). Therefore, biopesticides have the potential to replace synthetic pesticides as they have generally short persistency on plants and high selectivity when used as extracts (Bakkali et al., 2008). The purpose of our study was to evaluate the bioactivity of NOVOSECT SC21® (Mix of metabolites of the Bacillus F.D. 777), ATO BED BUGS® and NEO-BOOST® (Powdered Peracetic Acid & Bio Silicate) on the infestation and mortality level of *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani* infection on tomatoes in an open field in Hrajel Lebanon.

2. Material and Methods

2.1 Experimental Site

The experiment was carried out during the 2019-2020 cropping season from June to September at a private tomato field in Hrajel, Mount Lebanon (33°01′41″N; 35°79′49″E), 1400 m above sea level. Water was available from two sources: artesian well and a lake which accumulates spring water. The irrigation system was a drip system.

2.2 Plant Source and Supply

Tomato seeds, var. Maysa (Antagro s.a.l.), with a germination rate 92-96% and a 99.9% purity were used in the research. This variety has a dense to medium foliage and is resistant to tomato spotted wilt virus (TSWV) caused by thrips. The seeds were planted in the nursery of the Faculty of Agriculture (April 27, 2020), and the seedlings were transplanted into the field in the mid of June (June 15, 2020) having a height between 12 and 15 cm. Two hundred and fifty-two tomato plants were evaluated for the efficiency of the biopesticides ATO BED BUGS®, NOVOSECT SC21®, and NEO-BOOST® (Powdered Peracetic Acid & Bio Silicate) in controlling two major pests encountered in Mount Lebanon: *Tuta absoluta* and *Liriomyza trifolii*, and the fungal disease *Alternaria solani*. In the field, the experimental design was randomized complete block design RCBD) with three replications, three treatments and the untreated control. The biopesticide treatments include (A) ATO BED BUGS® 1 L/200 L, (B) bacteria NOVOSECT SC21® 0.5 L/200 L, and (D) NEO-BOOST® (Powdered Peracetic Acid & Bio Silicate) 1 kg/200 L. The fourth treatment was the untreated control (C). The products used originated from Atomes F.D. Inc., Canada. Two hundred fifty-two tomato plants were planted in 3 blocks. Each block of 5.8 m length, was consisted of 12 plots distributed into two rows. Each plot contained 7 plants distanced 0.5 m. The distance between rows and between blocks was equal to 1 m. There were 2 m unplanted from each side of the field to prevent the existence of any infected plant or contamination from the neighboring area (Figure 1).

Figure1. Experimental field (Hrajel, 2020)

2.4 Field Trials

The experiment included two major field trials. The first set of field trials was conducted to assess the abundance and diversity of two pests: *Tuta absoluta*, *Liriomyza trifolii* and fungal presence: *Alternaria solani*. The second

set of field trials was conducted in order to evaluate the biopesticides effects on larval toxicity and fungal infestation level.

2.5 Collected Data

The pattern of infestation and resultant damage varied not only from species to species but within species, depending on life cycle stage: egg, larvae, pupa and adult. Signs of insect infestation were:

- ✓ Live insects mostly found inside and on plant parts, within leaves;
- ✓ Insect remains including whole carcasses body parts and cast stains;
- ✓ Frass, droppings and tunnels.

Direct observations in the field have advantages can help identify the pest, possible discovery of predators or any other predator previously not believed to be a predator, and the number of insects per unit of area and time. The number of individuals found and the population abundance were collected and compared.

2.5.1 Effect of Biopesticides on the Insect Population Infestation and Fungal Infection

One day prior to the first pulverizations of the ATO BED BUG®, NOVOSECT SC21®, NEO-BOOST®, and the control), a first lecture was done in order to indicate *T. absoluta*, *L. trifolii* and *A. solani* symptoms on the whole plant (Figures 2-4). Two plants were chosen from each plot. The first treatment was applied on August 24, 2020, using a knapsack sprayer with a capacity of 20 L. A second, third and a fourth observation were completed after 24, 48, and 72 hr. The purpose of the observations was to determine if the level of infestation by these pests increased, what new symptoms appeared, the number of living larvae, the amount of frass, etc. In the case of *A. solani*, the level of infection was measured by observing the emergence of new symptoms (dark concentric rings) on the whole plant other than that marked before in the previous observation.

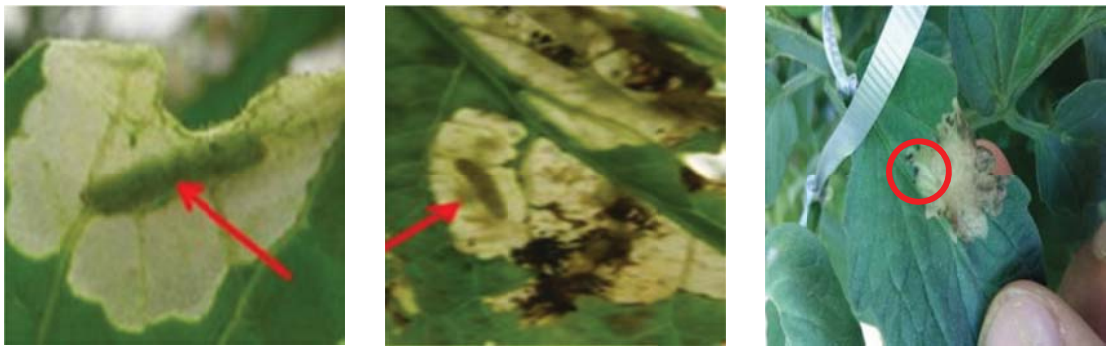

Figure 2. Symptoms of *Tuta absoluta* infestation (live larvae, frass) (Hrajel, 2020)

Figure 3. Symptoms of *Liriomyza trifolii* infestation (live larvae, frass) (Hrajel, 2020)

Figure 4. Symptoms of *Alternaria solani* infection (Hrajel, 2020)

2.5.2 Effect of Biopesticides on the Larval Mortality of *Tuta absoluta* and *Liriomyza trifolii*

During the second pulverization on the 13 September 2020, three plants per plot were chosen, and from each plant, three leaves presenting *Tuta* and/or *Liriomyza* symptoms were indicated one day before the treatment. 24, 48 and 72 hr after the pulverization, the symptoms were observed to evaluate the mortality of those two insects (Figure 5). The mortality of the *T. absoluta* larva was identified through the observation of the larvae mine. If the larvae color changes pale from green color, then from brown or black. This means that the larvae were dead. The dead larvae and insects became extremely withered because of dehydration (Kaoud, 2014). The mortality of *Liriomyza trifolii* was identified by observing the color and the size of the larvae tunnels on the leaf. If the tunnels remain small and their color changed from green to brown, this was an indication that the larvae were dead. The mortality level of *Tuta absoluta* and *Liriomyza trifolii* larvae was calculated using the Abbot's formula $[(Ca - Ta)/Ca] \times 100$, where Ca represents the number of live control larvae after treatment and Ta the number of live test larvae after the treatment.

Figure 5. Different symptoms of *Tuta absoluta* and *Liriomyzae trifolii* on the leaf and the fruit (Hrajel, 2020)

2.6 Statistical Analysis

The statistical analysis was computed using IBM SPSS statistics 16.0, for the Anova analysis, Duncan test and T-test. The design of the experiment was based on RCBD design (Randomly Complete Block Design).

3. Results

3.1 Effect of Biopesticides on *Alternaria solani* Infection

The results obtained showed that there is no significant difference between the level of infection between 24 and 48 hr ($F = 9.761$, $P = 0.794 > 0.05$). Besides, using the paired sample statistics between the level of infestation of *A. solani* at 48 and 72 hr after the treatment shows that the means are 1.041±1.96 and 0.90±0.22, respectively at 48 and 72 hr, with a very strong correlation between these times (correlation = 0.906) which indicates that there is no significant difference between the level of infection at 48 and 72 hr (Table 1).

Since there is no significant difference between the infection levels of *A. solani* among time, we will be based later on the results obtained at 24 hr after the treatment to indicate the efficiency of each treatment.

In addition, ANOVA test indicates that there is a significant difference among the 4 different treatments at 24 hr.

Table 1. Average infestation level of *Alternaria solani*

	Pretreatment	24 hr	48 hr	72 hr	Average	Standard deviation
ATO BED BUG	2.22	0.77	0.833	0.5	1.08075	0.669
NOVOSECT SC21®	2.167	0.77	0.611	0.611	1.03975	0.654
NEO-BOOST	2.389	0.611	0.666	0.389	1.01375	0.80
Control	2.5	1.888	2.05	2.11	2.137	0.224

Table 1 shows that the three products used had approximately similar effects (ATO BED BUG® 1.08±0.66; NOVOSECT SC21®: 1.03±0.65 and NEO-BOOST®: 1.01±0.8) on reducing the emergence of new *Alternaria* symptoms on the whole plant, similar results were obtained by the Duncan test.

3.2 Effect of Biopesticides on Tuta absoluta Infestation

The results obtained using T-test between 24 and 48 hr, indicate a reduction in *Tuta absoluta* level of infestation/plant: the mean after 24 hours is 1.36±0.49, while the mean at 48 hr is 0.91±0.22. The paired samples correlation indicates that there is a moderate correlation between 24 and 48 hr ($r = 0.547 < 0.8$), which means that there is a moderate relationship between the reduction of *Tuta absoluta* level of infestation at 24 and 48 hr with a significant difference ($P = 0.037 < 0.05$). The decrease of the level of infestation by *Tuta absoluta* is greater at 48 hr than its decrease at 24 hr after the treatment.

In addition, there is no significant difference between the level of infestation by *Tuta absoluta* at 48 and 72 hr after the treatment, with a mean of infestation equal to 0.91±0.22 and 1.08±0.23, respectively, and a very strong correlation and statistical relation between these two times ($r = 0.880$) (Table2).

ANOVA test showed that there is a highly significant difference between the efficiency of the tested groups at 48 hr and 72 hr after the treatment, $F = 28.68$, $P = 0.000$; $F = 42.50$, $P = 0.000$ respectively.

Table 2. Average infestation level of *Tuta absoluta*

	Pretreatment	24 hr	48 hr	72 hr	Average	Standard deviation
ATO BED BUG	3.389	1.5	0.666	0.999	1.6385	1.053
NOVOSECT SC21®	3.278	0.944	0.277	0.055	1.1385	1.277
NEO-BOOST	3.278	0.778	0.611	1.055	1.4305	1.078
Control	2.555	1.777	2.111	2.222	2.16625	0.277

Table 2 shows that NOVOSECT SC21® was the best in controlling the emergence of new *Tuta absoluta* symptoms on the whole plant at 72 hours after the treatment (1.138±1.27) followed by ATO BED BUG® and NEO-BOOST® respectively (1.63±1.05; 1.43±1.07). The Duncan test showed similar results.

3.3 Effect of Biopesticides on the Larval Mortality of Tuta absoluta

The T-test between 24 and 48 hr after the treatment shows that the mean of the level of *Tuta absoluta* mortality/leaf are 0.71±0.15 and 0.7±0.22 respectively, with a very strong correlation value (0.949), and no significant difference (Table 3).

The ANOVA test showed that there is a highly significant difference between groups among time: at 24 hr $F = 30.59$, $P = 0.000$; at 48 hr $F = 41.1$, $P = 0.000$; at 72 hr $F = 563.35$, $P = 0.000$). NOVOSECT SC21® showed to be the most effective with a mortality level reaching 100% at 72 hr after the treatment. While ATO BED BUG® has been more effective than NEO-BOOST ® among time. Similar results were also obtained using the Duncan test.

Table 3. Mortality percentage of *Tuta absoluta* larvae after the treatment

	24 hr	48 hr	72 hr
ATO BED BUG	12.75%	82.77%	79.36%
NS SC21	83.11%	92.40%	100%
NEO-BOOST	24.08%	79.10%	87.80%

3.4 Effect of Biopesticides on Liriomyza trifolii Infestation

The T-test between the level of infestation by *Liriomyza trifolii* after 24 and 48 hr of the treatment, showed that the mean of the level of infestation by *Liriomyza*/plant is equal to 0.65±0.065, and 0.93±0.13 at 24 and 48 hr after the treatment, respectively. The correlation between the level of infestation/plant at 24 and 48 hr after the treatment is weak (0.369 < 0.8), which mean that there is a significant difference between the level of infestation by *Liriomyza trifolii* after 24 and 48 hr from the treatment. While the T-test between the level of infestation of *Liriomyza trifolii* 48 and 72 hr after the treatment is respectively 0.93±0.132 and 0.58±0.11, with a highly significant difference between the level of infestation by *Liriomyza trifolii* after 48 and 72 hr (Table 4).

The ANOVA test showed that there is no significant difference among groups at 24 and 48 hours after the infestation: at 24 hr F = 1.149, P = 0.387; while at 48 hr F = 3.479, P = 0.07.

Table 4. Average infestation level of *Liriomyza trifolii* after the treatment

	Pretreatment	24 hr	48 hr	72 hr	Average	Standard deviation
ATO BED BUG	2	0.583	0.722	1.167	1.118	0.553
NOVOSECT SC21®	1.666	0.5	0.778	0.333	0.81925	0.514
NEO-BOOST	1.833	0.791	0.722	0.5	0.9615	0.514
Control	1.499	0.75	1.5	1.111	1.215	0.311

Table 4 shows that the products used had approximately the same effect on controlling the emergence of new *Liriomyza trifolii* symptoms on the whole plant (ATO BED BUG® 1.118±0.55; NOVOSECT SC21® 0.81±0.51; NEO-BOOST® 0.96±0.51). Similar results were obtained using the Duncan test.

3.5 Effect of Biopesticides on Liriomyza trifolii Larval Mortality

The T-test between 24 and 48 hr after the treatment showed that the mean of *Liriomyza trifolii* dead larvae/leaf has decreased 0.84±0.14 and 0.71±0.23 respectively with a high correlation value (0.889) indicating a strong relation between the level of mortality after 24 and 48 hr from the treatment, with no significant difference among time (0.32 > 0.05) (Table 5).

ANOVA test showed that there is a highly significant difference between groups at 24 and 48 hr after the treatment: at 24 hr F = 1.149±0.387; at 48 hr F = 3.479±0.070.

At 48 hr after the treatment, NOVOSECT SC21® and ATO BED BUG® induced the highest mortality, 90.83% and 88.97%, respectively (Table 5). The same results were observed at 72 hr, wherethe mortality for NOVOSECT SC21®, ATO BED BUG®, and NEO-BOOST, were 98.88%, 98.60%, and 88.68%, respectivitely. Similar results were observed using the Duncan test.

Table 5. Mortality percentage of *Liriomyza trifolii* larvae after the treatment

	24 hr	48 hr	72 hr
Ato BedBug	78.35%	88.97%	98.60%
NOVOSECT SC21®	73.59%	90.83%	98.88%
NEO-BOOST	32.01%	76.10%	88.68%

4. Discussion

Biopesticides such as clove oil (eugenol), BT F.D. 777 are excellent alternatives to synthetic insecticides as a means to reduce residues and protect the environment (Isman & Machal, 2006). The objective of our research was to evaluate the effect of ATO BED BUG®, NOVOSECT SC21® and NEO-BOOST® on the level of infestation and mortality of *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani*.

4.1 Effect of Biopesticides on the Level of Tuta absoluta Infestation

The results showed that the effect of the treatments was completely opposite from the control: the resistance level of *Tuta absoluta* population decreased with time showing the best results of efficiency at 48 hr after the treatment. At 24 hr, biopesticides and the control showed same effect, while at 48 hr after the treatment, the three biopesticides (ATO BED BUG®, NOVOSECT SC21® and NEO-BOOST) had the same opposite effect as the control. While at 72 hr after the treatment, NOVOSECT SC21® was the most efficient on reducing the level of

infestation by *Tuta absoluta,* followed ATO BED BUG® and NEO-BOOST®. Through time, ATO BED BUG® was the most efficient in reducing the infestation level and its activity was optimal after 48 hr from the treatment, time needed for the larvae of *Tuta absoluta* to ingest NOVOSECT SC21® metabolites. These results agree with the results of Derballa et al. (2012), who reported that the Bt metabolites having tpotential insecticidal activity against Lepidopterans pests. ATO BED BUG was also effective in reducing the infestation level by *Tuta absoluta*, but its efficacy decreased with time due to its volatility. Similar results were observed by Ebadah et al. (2006) showing a reduction of *Tuta absoluta* infestation between 50-60% when treated with clove oil under semi field conditions. Similarly, Mouawad et al. (2013) recorded that clove oil caused highly reduction percentage of penetration and accumulative mortality of larvae and caused ovipositional deterrence reaction towards adult stage of *T. absoluta* under laboratory conditions.

4.2 Toxicity of Biopesticides on Larvae of Tuta absoluta

NOVOSECT SC21® was the most effective in controlling *T. absoluta* by causing 92.40% mortality at 48 hr after the treatment: the larvae ingest the crystal inclusions (Cry toxins) which will be dissolved and activated in the alkaline environment of the insect gut, where it binds to specific receptors and cause the lyse of larvae midgut (Bravo et al., 2005). Similar observation was obtained with Derbalah et al. (2012) where a combination of *Bacillus thuringiensis* filtrate and Indoxacarb, have showed a reduction of larvae and mine blotch count in treated plants, and where Bt filtrates has exhibited satisfactory effectiveness against *T. absoluta* in greenhouses. ATO BED BUG® was effective in increasing the mortality of *T. absoluta* larvae (82.77% at 48 hr after the treatment), similarly, Mouawad et al. (2013) affirmed that clove oil gave satisfactory results against *T. absoluta*.

4.3 Effect of Biopesticides on the Level of Liriomyza trifolii Infestation

The effect of all the biopesticides was optimal at 48 hr after the treatment. Similar results were reported by Ebadah et al. (2016) where the efficiency of three recommended insecticides (Acetamiprid 20% SP, Chlorpyrifos 48% and Lambda-cyhalothrin 5% EC) were compared to two natural oils (clove oil and bitter orange) against some of tomato insects (*Liriomyza trifolii*, *Tuta absoluta* and *Bemisia argentifolii*) under semi field conditions. The study showed a 48.7% reduction in tunnels after being treated with clove oil by between 3 and 5 days compared to a 52.5% reduction of tunnels after being treated with chlorpyriphos.

4.4 Biopesticides Toxicity Against Liriomyza trifolii Larvae

Ebadah et al. (2016) reported that clove oil caused 56.8% mortality of *L. trifolii* compared to 59.2% and 69.1% mortality when treated with Chlorpyrifos and Lambda-cyhalothrin, respectively after three days. While after 5 days of the treatment, the mortality percent was increased to 61.4% when treated with clove oil compared to 43.7% by Acetamiprid and 40.1% by bitter orange. Based on the 215 day mortality mean, clove oil was the produced the greatest mortality of *L. trifolii* (60.5%). These results are in agreement with those obtained by Sabbour and Abd-El-Aziz (2010), who reported that clove oil and mustard revealed a strong repellent activity after 7 days (71 and 89%, respectively) against *Bruchidius incarnates*. Clove oil was also effective against other Dipteran's insects, such as *Anopheles dirus* mosquitoes (Trongtokit et al., 2005) and clove oil displays an insecticidal activity against this mosquito (Chaeib et al., 2007a). Cikman et al. (2006) reported that Bt is effective in controlling the larvae of *L. trifolii* and should be treated once every 2-3 weeks for effective control. According to Cikman (2006), Bt treated leaves have significantly fewer live larvae than non-treated leaves and that during the whole production period (15 weeks) the number of live larvae was still fewer than the number at which treatment is recommended (4-5 larvae per leaf). Ramirez-Godoy et al. (2018) showed that soil and foliar silicate applications enhanced plant resistance against the Asian Citrus Psyllid in Tahiti lime. Our results suggest that foliar NEO-BOOST applications should be considered by growers because it has an impact on Liriomyza populations.

4.5 Effect of Biopesticides on Alternaria solani Infection

ATO BED BUG® was efficient in controlling *Alternaria solani*. According to Manohar et al. (2001), the phenolic components of clove oil: eugenol and carvacrol possess fungicidal characteristics on the cellular membrane. Also, clove oil has shown an antifungal activity against *Candida albicans* and *Trichophyton mentagrophytes* (Tampieri et al., 2005). Other studies have shown that a mixture of clove with concentrated sugar solution produced a strong fungicidal effect by reducing the fungi inoculum size (Nunez et al., 2001). Also, Pawar et al. (2006) affirmed that clove oil inhibited the growth of *Asparagillus niger*. The activity of clove oil against fungi is exerted on the cellular membrane and depends on the presence of aromatic ring and the presence of free phenol hydroxyl group and the lipophilic features of the components present in the oil (Tampieri et al., 2005; Cox et al., 2001; Chaeib et al., 2007b).

5. Conclusion and Recommendation

Our research indicates that NOVOSECT SC21® and ATO BED BUG® has potential for controlling *Tuta absoluta* and *Liriomyza trifolii* by reducing the infestation level and increasing the larval mortality. Additional formulation research should be conducted to optimize the benefits of each material. This formulation research should involve investigating the impact of surfactants or other additives of the efficacy of the biopesticides and maximize their effective longevity once applied to the plants. Biopesticides might compete and replace conventional pesticides for the control of tomato leafminer *Tuta absoluta*, *Lyromyza trifolii* and *Alternaria solani* fungus. An economical study should be conducted in order to provide a better understanding of these biopesticides as alternatives to the conventional chemicals used by Hrajel regional farmers. Thus, these biopesticides are highly recommended to be implemented with other types of control measures as biological control agents or other biorationals in sustainable agro-ecosystems, such as organic farming and in integrated pest management (IPM) programs for *Tuta absoluta*, *Liriomyza trifolii* and *Alternaria solani*.

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Inheritance and Allelic Relationships of *Alectra vogelii* Benth. Resistance Genes in Cowpea Genotypes B301 and KVx414-22-2

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Abstract

Alectra vogelii Benth. is the second most important parasitic weed in cowpea [*Vigna unguiculata* (L.) Walp.] production in Burkina Faso. Several resistant varieties to this weed have been identified in the country among which are B301 and KVx414-22-2. The inheritance and allelic relationships of the resistance genes in the two varieties have not been studied with *A. vogelii* strains in Burkina Faso. The objective of this study was to determine the inheritance and allelic relationship of the resistance genes in B301 and KVx414-22-2. To determine the inheritance of the genes for resistance, the resistant varieties (B301 and KVx414-22-2) were each crossed to a susceptible variety IT82D-849 to generate F_1 and F_2 populations. For the allelic relationship study the two resistant genotypes were crossed among themselves to generate F_1 and F_2 offspring. The parents and their F_1 and F_2 progenies were screened in artificially infested pots with *Alectra* seed in a screen house at Kamboinsé Research Station in Burkina Faso. Resistance/susceptibility of genotypes was assessed by recording the number of emerged *Alectra* shoots. The data were subjected to the Chi-Square goodness-of-fit test for one, two and three genes segregation ratios. The results revealed that two independent dominant genes confer resistance in the variety B301 and a single dominant gene confers resistance in variety KVx414-22-2. The single dominant gene in KVx414-22-2 is non-allelic to the two genes in B301. The two resistance genes in variety B301 have already been named *Rav1* and *Rav2* whilst *Rav3* is the name of the resistance gene in variety IT81D-994. Therefore, we propose the symbol *Rav4* as the name for the resistance gene in variety KVx414-22-2.

Keywords: *Alectra vogelii*, allelic relationship, dominance, resistance gene, *Vigna unguiculata*

1. Introduction

Cowpea (*Vigna unguiculata* L. Walp.) is one of the major grain legumes produced in semi-arid and arid areas of the world. The high protein quality of this legume for both human and animal nutrition is acknowledged worldwide. Its production also generates cash income for stakeholders.

Africa is by far the largest cowpea producing continent in the world. However, the African continent is also the area where the most devastating cowpea production constraints prevail (drought, insect pests, diseases and parasitic weeds) (Singh & Allen, 1979; Horn & Shimelis, 2020). Both abiotic and biotic constraints very often lead to significant yield losses. Parasitic weeds *Alectra vogelii* and *Striga gesnerioides* are two major cowpea production constraints in Africa. *Alectra vogelii*, though less widely studied than *Striga gesnerioides*, is widespread and causes severe damage to cowpea production (Mohamed et al., 2006; Kabambe, Tembo, & Kazira, 2013). Yield losses ranging from 80% to 100% due to *A. vogelii* infestation have been reported (Mbwaga, Hella, Mligo, Kabambe, & Bokosi, 2011; Kabambe et al., 2013).

Several control measures (cultural practices and chemical control) have been used with ineffective results because of their cost, technical difficulties and inaccessibility. Therefore, genetic resistance remains the most efficient, affordable and environmentally friendly method for controlling this weed (Rubiales et al., 2006). Varieties conferring resistance to *A. vogelii* have been identified. However, the inheritance of the resistance in most of these varieties is yet to be investigated. (Atokple, Singh, & Emechebe, 1993) and (Singh et al., 1993) reported two independent dominant genes, namely $R_{av}1$ and $R_{av}2$ in landrace B301 from Botswana. However,

this variety does not have farmers' preferred traits (large seed size, rough and white seed) for the Burkina Faso market. It has small, smooth, brown seeds controlled by alleles that are dominant over those for large white seed (Noubissié et al., 2011). Therefore, using B301 as a donor parent for *Alectra* resistance while improving for farmers' preferences will slow down the selection process. Nevertheless, it remains a good donor parent because it combines resistance to both *Alectra* and *Striga*. It was also reported that a single dominant gene which was given the symbol, *Rav3*, confers resistance to *Alectra* in the variety IT81D-994 (Atokple, Singh, & Emechebe, 1995; Kouakou et al., 2009) which has farmers' preferred traits but this gene does not confer full resistance to *Alectra* (Atokple et al., 1995; Kouakou et al., 2009; Dieni et al., 2018). New sources of resistance to the weed, including B301 and KVx414-22-2, have been identified in Burkina Faso through both screen house and field screenings (Dieni et al., 2018). In addition to *Alectra* resistance, the variety KVx414-22-2 possesses some farmers' accepted traits. However, the inheritance patterns of the resistance gene(s) in this variety and the allelic relationships with those in variety B301 have not been studied. The objectives of this study were to (i) determine the inheritance patterns of the resistance in the varieties B301 and KVx414-22-2, and (ii) determine the allelic relationships between the genes conferring resistance to *Alectra vogelii* in these varieties.

2. Materials and Methods

2.1 Population Development

The genetic material used in this study comprised a susceptible genotype, IT82D-849 and two resistant genotypes, B301 and KVx414-22-2 (Dieni et al., 2018) used as parents. These parents were used in two sets of crosses. On one hand, the susceptible parent was crossed with each of the resistant parents to produce F_1 families (F_1 IT82D-849/B301 and IT82D-849/KVx414-22-2). The F_1 plants were self-pollinated to generate F_2 progenies (F_2 IT82D-849/B301 and IT82D-849/KVx414-22-2). On the other hand, the resistant parents were crossed between themselves to develop F_1 (B301/KVx414-22-2) and F_2 offspring (B301/KVx414-22-2) through self-pollination of the F_1 individuals. Overall, three populations of F_1 and F_2 progenies were developed from the three crosses.

2.2 Experimental Management and Data Collection

The three parents IT82D-849, B301, KVx414-22-2 and their F_1 and F_2 offspring were used in this study. Ten (10) individuals of each parent as well as the F_1 populations were screened in a screen house at Kamboinsé Research Station for their reaction to *Alectra vogelii*. For each of the three F_2 populations two hundred (200) individuals were evaluated in the same experiment.

The experiment was conducted in screen house at Kamboinsé Research Station from June to August 2017. Plants were screened in plastic pots of ten (10) L filled with 12 kg of sterile soil. The pots were infested with *Alectra vogelii* seeds, collected from an *Alectra* infested field in Koupela (Burkina Faso) in October at the end of the 2014 rainy season. The seeds were dried under shade and sieved with a suitable (250-300 μm) mesh sieve. The sieved seeds were stored at room temperature until use. Each pot was infested with about 1,000 *Alectra vogelii* seeds based on the recommendations of Musselman and Ayensu (1983) and Magani et al. (2008). The preconditioning of the *Alectra vogelii* seeds consisted of watering the infested pots for two weeks in order to break seeds dormancy and ensure their uniform germination. This period of time has been reported to be optimal for preconditioning *Alectra* seeds (Magani et al., 2008). A single cowpea seed was planted per pot per genotype. Each parent and F_1 population was planted in ten pots; 200 pots were used for each of the F_2 populations making a total of 660 pots. The pots were kept moist by watering when it was necessary. From six (6) to ten (10) weeks after cowpea planting, pots were carefully checked to record the number of *Alectra vogelii* shoots that emerged. Plants which supported *Alectra* emergence were considered as susceptible; otherwise they were classified as resistant.

2.3 Data Analysis

The data collected were subjected to Chi-square "goodness of fit" test at 5% level of significance. For the inheritance studies, the segregation ratios were compared to Mendelian segregation ratios for one (3R:1S) and two genes (15R:1S). The hypothesis tested was that the observed segregation patterns follow Mendelian ratios. This hypothesis was rejected when the chi-square value was significant (the calculated chi-square was greater than the theoretical chi-square) otherwise it was accepted (the calculated chi-square was less than the theoretical chi-square).

To determine the allelic relationship between the genes for resistance in the two parents, the Mendelian's ratios for two dominant genes (15R:1S) and three dominant genes (63R:1S) were tested. The assumptions underlying this analysis are as follows:

(i) If two dominant genes A and B confer the resistance and each of the homozygous parents carry different resistance gene, then the parents can be AAbb and aaBB respectively. F_1 progenies derived from a cross between them will be AaBb which are resistant because of the presence of dominant A and B genes;

(ii) If the genes are allelic, then both the first (F_1) and the second (F_2) generations derived from a cross between these two resistant parents are expected to comprise only resistant progenies. In this case the parents would have been AA and AA respectively.

(iii) If they are not allelic segregation ratio in the F_2 generation will be consistent with the normal Mendelian ratio: 9A_B_:3A_bb:3aaB_:1aabb. All progenies carrying at least a dominant allele are expected to be resistant whilst susceptible progenies are those with homozygous recessive alleles (aabb) to give a ratio of 15R:1S.

(iv) If three dominant genes A, B and C confer the resistance and one of the homozygous parents carries two different resistance genes all different from the resistance gene in the second homozygous parent, then the parents can be AABBcc and aabbCC or other suitable combinations where one homozygous parent has two dominant genes at two loci but recessive on the third and the other homozygous parent has one dominant gene corresponding to the recessive locus in the in the first parent and recessive genes corresponding to the dominant loci of the other parent. A cross between them generates F_1 progenies of fully heterozygous genotype AaBbCc which are resistant because of the presence of dominant A, B and C genes.

(v) If the genes are allelic, then a cross between two resistant individuals would generate only resistant progenies in both the F_1 and F_2 generations. In this case the genotypes of the parents could be AABB and aaBB or AABB and AAbb.

(vi) If they are not allelic, segregation ratio in the F_2 generation will be in agreement with the normal Mendelian ratio: 27A_B_C_:9A_B_cc:9A_bb_C_:9aaB_C_:3A_bbcc:3aaB_cc:3aabbC_:1aabbcc. All progenies carrying at least a dominant allele are expected to be resistant whilst susceptible progenies are those with homozygote recessive alleles (aabbcc) to give a ratio of 63R:1S.

The equation for the Chi-square is as shown below:

$$\chi^2 = \sum_{i=1}^{k} (O_i - E_i)^2/E_i \quad (1)$$

Where, χ^2 = Chi-Square value; O_i = observed frequency of class i; E_i = expected frequency of class i; k = number of classes.

3. Results

3.1 Inheritance Patterns of the Resistance Genes

Results from the screening of the non-segregating populations are as follows: all the 10 individuals of the susceptible parent (IT82D-849) screened supported severe *Alectra* infestation while the resistant parents (B301 and KVx414-22-2) as well as both F_1 populations were free of *Alectra* infestation.

Among 200 F_2 progenies derived from a cross between IT82D-849 and B301, 18 individuals were susceptible (S) to *Alectra* while 182 were resistant (R). In the second F_2 population (IT82D-849/KVx414-22-2), 62 individuals out of 200 supported *Alectra* shoots emergence while 138 were resistant. The segregation ratios are presented in Table 1. The chi-square "goodness-of-fit" test for segregation patterns of the F_2 population from IT82D-849/B301 showed a good fit to a 15R:1S ratio (chi-square value 2.58, $P < 0.0.05$) with 182R:18S observed against expected values of 187.5R:12.5S. The segregation patterns in the F_2 population from IT82D-849/KVx414-22-2 was a good fit to a 3R:1S ratio (chi-square value 3.09, $P < 0.05$) with 138R:62S against expected values of 150R:50S (Table 1).

Table 1. Segregation patterns of the inheritance of *Alectra vogelii* resistance in two F_2 populations of cowpea

Cross	Total	Observed frequencies		Expected Frequencies		Ratio	χ^2	p-value
		R	S	R	S			
IT82D-849/ B301	200	182	18	187.5	12.5	15:1	2.58	**0.108**
IT82D-849/KVx414-22-2	200	138	62	150	50	3:1	3.09	**0.079**

Note. R: resistant, S: susceptible, χ^2 = Chi-Square value.

3.2 Allelic Relationship

The segregation patterns of F_2 progenies derived from the cross between the two resistant genotypes (B301/KVx414-22-2) are presented in Table 2. For the 200 F_2 plants tested only four were susceptible to *Alectra vogelii* and 196 were resistant. The χ^2 goodness-of-fit test was a good fit to a 63R:1S ratio (Chi-square value 1.63, $P < 0.05$) (Table 2). Therefore, the F_2 population segregated into a ratio 63 resistant: 1 susceptible.

Table 2. Segregation ratios of the allelic relationships of *Alectra vogelii* resistance in F_2 population derived from B301 and KVx414-22-2

Cross	Total	Observed frequencies		Expected frequencies		Ratio	χ^2	p-value
		R	S	R	S			
B301/KVx414-22-2	200	196	4	192.59	7.41	63:1	1.63	**0.202**

Note. R: resistant, S: susceptible, χ^2 = Chi-Square value.

4. Discussion

The resistant parents (B301 and KVx414-22-2) and all F_1 progenies derived from the different crosses were resistant to *Alectra vogelii*. The susceptible parent (IT82D-849) showed high degree of infestation confirming its susceptibility. The homogeneity of the F_1 progenies for resistance to *Alectra vogelii* demonstrated that at least one dominant gene is responsible for the resistance in each of the varieties. A dominant inheritance patterns was reported for B301 (Atokple et al., 1993; Singh et al., 1993).

The F_2 progenies derived from the cross between IT82D-849 and B301 segregated into a ratio of 15R:1S confirming that two dominant genes confer the resistance to *Alectra vogelii* in B301as reported by Atokple et al. (1993) and Singh et al. (1993). The F_2 offspring from IT82D-849/KVx414-22-2 assorted into a ratio of 3R:1S. The segregation ratio conforms with the Mendelian segregation ratio for one dominant gene conferring resistance to *A. vogelii*. Therefore, a single dominant gene governs the resistance in KVx414-22-2. Single dominant gene inheritance patterns was reported for *Alectra* resistance in IT81D-994 (Atokple et al., 1995; Kouakou et al., 2009). Several dominant genes (*Rsg1*, *Rsg2*, *Rsg3* and *994-Rsg*) have been reported for cowpea resistance to *Striga gesnerioides* in different genotypes including B301, IT82D-849 and IT81D-994; among them Rsg_1 and Rsg_2 have been shown to be allelic (Atokple et al., 1993, 1995; Ouedraogo et al., 2001; Singh et al., 1993; Tignegre, 2010).

For the allelic relationship study, the observed segregation ratio of the cross between the two resistant genotypes was similar to the inheritance patterns of a character governed by three independent dominant genes. The independent inheritance patterns of the two genes conferring resistance in B301 has been reported by Atokple et al. (1993) and confirmed by the present study. Thus, if one of these two genes was allelic to the resistance gene in KVx414-22-2, then the segregation ratio of the F_2 progenies derived from B301/KVx414-22-2 should be consistent with Mendelian's segregation patterns for two independent genes. However, this hypothesis was rejected ($p < 0.05$) because a ratio of 63 resistant to 1 susceptible indicated that the three genes were independent and located on three different chromosomes. Therefore, it could be concluded that the two genes conferring resistance to *Alectra* in B301 and the one responsible for resistance in KVx414-22-2 are not allelic. Non allelic relationship between the genes conferring resistance to *Alectra vogelii* in B301 and IT81D-994 has also been reported (Atokple et al., 1995). Therefore, the symbols Rav_1, Rav_2 and Rav_3 were proposed for the two resistance genes in B301 and the one gene in IT81D-994 respectively (Singh et al., 1993; Atokple et al., 1995). In contrast to the fully resistant varieties B301 and KVx414-22-2, the variety IT81D-994 is moderately resistant to the ecotype of *Alectra* from Koupela (Dieni et al., 2018). This genotype also showed differential reaction to *Alectra* in Nigeria (Singh et al., 1993; Omoigui et al., 2012). On the basis of these findings, the resistance genes in

genotypes KVx414-22-2 and IT81D-994 are considered to be different and non-allelic. Therefore, the symbol *Rav4* could be proposed for the resistance gene in KVx414-22-2. The variety KVx414-22-2 is an improved line from Burkina Faso possessing farmers' preferred traits (large and white seeds) but it is *Striga* and virus susceptible. Nevertheless, it can be an ideal donor parent for improving cowpea for both *Alectra* resistance and grain quality through backcross breeding.

5. Conclusion

The results of this study confirmed that the resistance to *Alectra vogelii* is conferred by two independent dominant genes in the genotype B301. On the other hand, the resistance is conferred by a single dominant gene in the variety KVx414-22-2. The three genes in the two varieties are non-allelic. The two genes in B301 have been named *Rav1* and *Rav2* and the gene present in IT18D-994 has been given the symbol *Rav3* as noted earlier. The symbol *Rav4* is therefore proposed to be the name of the resistance gene in genotype KVx414-22-2.

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Essential Oil Variation in Brazilian *Varronia curassavica* Jacq. in Response to Drying and Edaphoclimatic Conditions

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Abstract

Varronia curassavica Jacq. (Boraginaceae) is a native species of the Atlantic Forest with medical importance. This study determined the essential oil variation of 16 populations of *V. curassavica* of restinga in Santa Catarina, Brazil, in response to drying and edaphoclimatic conditions. The populations supplied essential oil with a content between 0.27 to 1.15% in the summer and from 0.33 to 1.12% in the winter. 41 chemical compounds were identified in the summer and 40 in the winter. The compounds were grouped into 4 chemical classes in each station studied. The common chemical constituents found in the essential oil of all populations and in both seasons were α-thujene, α-pinene, sabinene, α-humulene, (*E*)-cariophylene, spatulenol, mircene, allo-aromadendrene, β-sesquifelandreno and α-zingiberene. Cluster analysis using the nearest neighbor method based on Euclidean distance grouped the 16 populations into 3 groups in the summer and 8 groups in the winter. As the habitats have distinct pedological characteristics, we identified that pH, organic matter, sum of bases and base saturation are associated with the synthesis of (*E*)-caryophyllene, α-humulene, and allo-aromadendrene from populations.

Keywords: aromatic plant, chemical composition, phytochemical, restinga

1. Introduction

The Atlantic Forest is one of 25 recognized biodiversity hotspots globally; it is home to more than 19,000 species, of which 35% are endemic (Oliveira et al., 2019; Souza et al., 2021). Despite the remarkable endemism levels that make the Atlantic Forest one of the most distinct regions in the Neotropics (Ribeiro et al., 2011; Souza et al., 2020), little is known about the aromatic plants' potential genetic resources in this biome. Among the botanical species that occur in the restinga—an ecosystem associated with the Atlantic Forest biome and established on sandy soils of marine origin—*Varronia curassavica* Jacq. (synonym = *Cordia verbenacea* DC.) (Boraginaceae) is considered one of the main sources of molecules used in the treatment of inflammation, rheumatism, and ulcers (Passos et al., 2007; Roldão et al., 2008). This is due to the high diversity of secondary metabolites, specifically, the essential oils (EOs), which are synthesized and stored in the glandular trichomes present on the leaf surface (Feijó et al., 2014). In addition, the EO of this species stands out for being the first topical phytotherapeutic developed entirely in Brazil, with anti-inflammatory action (Nizio et al., 2015). With the commercial name of Acheflan® and launched by the Aché Laboratory in 2011, this phytomedicine has achieved prescription leadership in the medicinal plant segment, with over 1 million units sold, representing USD 8.1 million and accounting for 10% of industry revenue (Oliveira, 2017). Besides, *V. curassavica* has recognized efficacy by the Brazilian Health Regulatory Agency and appears in different official lists of the Brazilian Ministry of Health (Oliveira, 2017).

Pre-clinical studies with histamine-induced edema assays in mice have attributed to the sesquiterpene α-humulene the role in the anti-inflammatory effect of the EO from *V. curassavica* (Fernandes et al., 2007; Passos et al., 2007). However, the α-humulene content of this plant varies greatly (0.3-31.6%) (Marques et al., 2019; Queiroz et al., 2020), and environmental factors must be considered to choose the best time to obtain the

EO with the desired amount of the substance of interest. For example, the pharmaceutical industry demands that plants have a minimum content of 2.0% α-humulene to meet the quality standard (Magalhães, 2010).

Various environmental conditions influence the production of EOs; among them, the year-season stands out; when the season changes, plants perform physiological changes in their metabolism to amplify CO_2 uptake and water and nutrient cycling, thus reflecting in the increase or decrease in the content and/or relative percentages of the compounds present in EOs (Dehsheikh et al., 2019). Some authors attribute that temperature can alter EO production via activation of thermosensitive enzymes involved in the mevalonic acid pathway, precursors of terpenes (Burbott & Loomis, 1967; Rahimmalek & Goli, 2013; De Almeida et al., 2016). Additionally, solar radiation can influence EO production directly or indirectly through increased plant biomass (Burbott & Loomis, 1967). EOs production is influenced by various environmental conditions, of which the seasons of the year stand out. Season change entails physiological changes in plant metabolism to amplify CO_2 uptake and water and nutrient cycling, reflecting the increase or decrease in the content and/or relative percentages of compounds present in EOs (Dehsheikh et al., 2019). Some studies have ascribed that temperature can alter EO production via activation of thermosensitive enzymes involved in the mevalonate pathway, a precursor of terpenes (Burbott & Loomis, 1967; Rahimmalek & Goli, 2013; De Almeida et al., 2016). Additionally, solar radiation can influence EO production directly or indirectly through increased plant biomass (Burbott & Loomis, 1967).

Previous studies have reported that the geographic location of plants along with the soil and climate conditions serve as modulators in EO production (Rahimmalek et al., 2017; Marques et al., 2019); thus, the same species may show differential EO production depending on the environment in which it is established. Similarly, that different genotypes can result in differential essential oil production, possibly due to the behavior of floral visitors that can induce gene flow among them (Hoeltgebaum et al., 2018). Another factor is that different plant phenological stages contribute to marked differences in EOs productivity (Bouyahya et al., 2019). However, regardless of the phenological stage at which the plant is harvested, this procedure is usually performed when the plant has high water content, and drying is the most widely used process to ensure the quality and stability of EOs after harvest.

Understanding the genotype × environment interaction may provide new insights for selecting *V. curassavica* matrices with potential pharmaceutical use, which should be coordinated with sustainable use practices of the species. Although *V. curassavica* has a wide geographic distribution along the coastal zones of the Santa Catarina state in Brazil (Bayeux et al., 2002), to our knowledge, there is no information about the chemical compounds of EOs found in these populations. In addition, the diversity of EOs among plants collected in natural habitats enables determining the collecting seasons, as well as the ideal growing conditions for domestication and improvement of the plants. In this sense, our objective was to determine the chemical variation of EOs from *V. curassavica* specimens collected from 16 native restinga populations in Santa Catarina, in response to drying and edaphoclimatic conditions.

2. Method

The experiments were conducted under field and laboratory conditions, during summer (February 2015 and 2016) and winter (September 2015 and 2016), to determine the influence of soil and climatic conditions on the OE content and chemical composition of *V. curassavica*

2.1 Characterization of the Collection Site

Detailed information about the collection site of the *V. curassavica* populations used in this study is detailed in Table 1. For the soil physicochemical analyses, six soil samples were collected at a depth of 20 cm at each location in April 2015. The samples from each location were pooled to form a composite sample and subsequently air-dried and sieved (1 mm). The fraction thinner than 1 mm was retained for physicochemical analyses. The potential of hydrogen (pH) was determined in a 1:1 soil-water volume ratio. Phosphorus (P) was extracted with Mehlich solution, and Aluminum (Al) was extracted with 1 mol L^{-1} potassium chloride (KCl). Organic matter (OM), base saturation (V), and the sum of bases (SB) were determined according to Silva (1999). The mean monthly values of temperature (Tp) and precipitation (Pp) in summer (February 2015 and 2016) and winter (September 2015 and 2016) were obtained from the climatological station of the Agribusiness Research and Rural Extension Company of Santa Catarina (*Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina*—EPAGRI).

2.2 Plant Material and Isolation of the Essential Oils (EOs)

Leaves from 16 populations of *V. curassavica* were collected along approximately 77 km of the Santa Catarina coast, between latitudes 26°22′22.2″S and 26°51′52.4″S, during the summer (February 2015 and 2016) and

winter (September 2015 and 2016). The populations were identified with codes according to the municipality where they are located (Table 1).

Table 1. Information on localities, soil and climate conditions, and size of native populations of *Varronia curassavica* collected in the restinga of Santa Catarina, Brazil

Locality[1]	Geographical coordinates	A[2] (m^2)	HP[3] (m)	HRF[4] (m)	pH[5]	P[6] (mg/dm^3)	OM[7] (%)	Al[8]	V[9]	SB[10]	TV[11] (°C)	TI[12] (°C)	PpV[13] (mm)[13]	PpI[14] (mm)
SF1	26°22′22.2″S; 48°34′25.0″W	13	0.47	0.17	5.8	6.3	0.2	0.00	54.14	1.07	24.7	17.7	256.3	107.7
SF2	26°23′48.4″S; 48°35′16.9″W	29	0.74	0.18	6.2	10.8	1.0	0.00	79.47	5.04	24.7	17.7	256.3	107.7
BS1	26°27′20.3″S; 48°35′16.9″W	15	0.94	0.27	5.5	16.8	0.2	0.00	62.84	1.35	24.8	18.2	256.3	107.7
BS2	26°28′30.4″S; 48°36′45.6″W	59	0.47	0.20	6.0	15.0	1.1	0.00	76.92	3.68	24.8	18.2	256.3	107.7
BS3	26°30′27.2″S; 48°37′56.2″W	11	0.51	0.18	5.7	9.3	1.0	0.00	65.70	2.10	24.8	18.2	256.3	107.7
BS4	26°31′48.6″S; 48°38′36.5″W	44	0.44	0.19	4.9	7.5	0.5	13.50	31.42	0.64	24.8	18.2	256.3	107.7
BV1	26°35′26.9″S; 48°40′10.8″W	9	0.57	0.19	4.7	5.9	0.7	30.47	34.72	1.60	22.5	16.2	202.0	99.3
BV2	26°36′10.7″S; 48°40′25.2″W	11	0.54	0.23	5.2	18.0	0.6	4.18	62.12	2.29	22.5	16.2	202.0	99.3
BV3	26°36′22.6″S; 48°40′29.5″W	6	0.54	0.22	6.2	11.0	1.1	0.00	73.96	3.40	22.5	16.2	202.0	99.3
PI1	26°43′38.6″S; 48°40′51.6″W	16	1.05	0.29	7.1	45.5	0.4	0.00	79.34	2.68	24.7	17.8	202.0	99.3
PI2	26°44′04.9″S; 48°40′49.6″W	8	1.23	0.26	5.5	5.0	1.1	0.00	69.66	3.68	24.7	17.8	202.0	99.3
PE1	26°45′48.3″S; 48°38′44.8″W	8	0.84	0.30	6.3	12.7	5.2	0.00	89.39	15.99	24.7	17.8	202.0	99.3
PE2	26°46′51.8″S 48°35′49.9″W	9	1.23	0.31	7.8	9.0	0.1	0.00	81.97	2.79	24.7	17.8	202.0	99.3
PE3	26°48′11.3″S; 48°35′49.7″W	10	1.08	0.29	8.0	13.2	1.2	0.00	91.52	6.46	24.7	17.8	202.0	99.3
NA1	26°51′28.9″S; 48°38′09.0″W	18	0.68	0.24	5.4	14.3	0.2	0.00	51.45	1.48	24.7	17.8	202.0	99.3
NA2	26°51′52.4″S; 48°38′15.0″W	26	0.84	0.27	5.2	12.2	0.3	6.70	53.75	1.39	24.7	17.8	202.0	99.3

Note. [1] SF1, São Francisco do Sul; SF2, São Francisco do Sul; BS1, Balneário Barra do Sul; BS2, Balneário Barra do Sul; BS3, Balneário Barra do Sul; BS4, Balneário Barra do Sul; BV1, Barra Velha; BV2, Barra Velha; BV3, Barra Velha; PI1, Balneário Piçarras; PI2, Balneário Piçarras; PE1, Penha; PE2, Penha; PE3, Penha; NA1, Navegantes; NA2, Navegantes. [2] Population area. [3] Average plant height. [4] Average height of the portion of branches with leaves. [5] Potential of hydrogen. [6] Phosphorus. [7] Organic matter. [8] Alumínio (% saturation in cation exchange capacity). [9] Percentage of base saturation. [10] Base saturation. [11] Average summer temperature. [12] Average winter temperature. [13] Average summer precipitation. [14] Average winter precipitation.

On the northern coast we studied populations from São Francisco do Sul (populations SF1 and SF2), Balneário Barra do Sul (BS1, BS2, BS3 and BS4) and Barra Velha (BV1, BV2 and BV3), while on the central-northern coast we studied populations from Balneário Piçarras (PI1 and PI2), Penha (PE1, PE2 and PE3) and Navegantes (NA1 and NA2) (Table 1). Populations SF1 in the far north and NA2 in the far south are equidistant in a straight line by approximately 55 km.

In the Atlantic Forest biome, the vegetation cover is inserted in the restinga associated ecosystem, whose ecological nature is conditioned to the vegetation complex established on sandy soils of marine origin. Each population consisted of a minimum number of 20 individuals. All individuals were selected within a maximum distance of 30 cm per work unit and collected on a single day. These populations were also characterized

according to the size of their range, average plant height, and the average height of the portion of branches with leaves (Table 1).

To obtain the EOs corresponding to the fresh leaves, samples of the collected populations were immediately taken to the laboratory and subjected to hydrodistillation in a Clevenger apparatus (Vidrolabor®, São Paulo, Brazil) for 3 h. On the other hand, for the EO obtained from the dried leaves, the plant material was previously kept under a forced air circulation oven (Fanem®, São Paulo, Brazil) at 40 °C until the mass remained constant before performing the hydrodistillation process mentioned above. The EO content was determined based on dry matter basis, with three replicates per treatment. Subsequently, the EOs obtained were separated with anhydrous sodium sulfate (Na_2SO_4) and kept refrigerated at -4 °C in amber flasks until the chemical analyses were conducted.

2.3 Chemical Analysis of Essential Oils (EOs)

GC-MS was carried out in an Agilent 6890 gas chromatograph coupled to an Agilent 5973 N mass selective detector. The GC was fitted with an HP-5MS fused capillary column (30 m × 0.25 mm × 0.25 μm film thickness) coated with 5% phenyl-95% dimethylpolysiloxane stationary phase. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Temperature programming was set to 60-240 °C at the rate of 3 °C/min, heated to 240 °C, and held at this temperature for 10 min. The injector temperature was kept at 250 °C. Essential oil samples were diluted to a 1% solution in dichloromethane, and 1.0 μL of the solution was injected with a split ratio of 1:20. The mass detector was operated in electron ionization mode (70 eV) at a 3.15 scan/min rate and a scan range of 40-450 Da. The transfer line was maintained at 260 °C, the ion source at 230 °C, and the analyzer (quadrupole) at 150 °C. For the quantification, the EOs were injected in an Agilent 7890A gas chromatograph fitted with FID operated at 280 °C. Hydrogen was used as the carrier gas at a flow rate of 1.5 mL/min, using the same column and conditions described above.

The quantification of each constituent was estimated by electronic integration of the FID signal with the corresponding peak area, which was determined based on the average of three injections (Tables 3 and 4). The identification of the oil's components was carried out by comparison of the mass spectra with those from commercial libraries and also by their linear retention indexes, after the injection of a homologous series of alkanes (C_8-C_{26}), under the same experimental conditions, compared to literature data (Adams, 2007).

2.4 Data Analyses

The EOs and α-humulene contents were submitted to the Shapiro-Wilk test to analyze the normality of the residuals and homogeneity of the variances. All data were subjected to analysis of variance (ANOVA), and the means were compared by the Scott-Knott method at 5% error probability. For the multivariate analysis, the Euclidean distance was estimated using the 'dist' function; the HCA was performed by the Unweighted Pair Group with Arithmetic Mean (UPGMA) method through the 'hclust' function, and the PCA was performed by the 'princomp' function. All of the functions belong to the 'stats' package. All data were analyzed in the "R" statistical software version 2.15.1 (R Development Core Team 2012).

3. Results

EO content from the fresh and dried leaves of *V. curassavica* differed with the seasons of the year. However, SF1 and BV3 populations showed no significant variations across seasons, with EOs contents ranging from 0.9 to 1.3% and 0.3 to 0.6%, respectively. In summer, EOs contents ranged from 0.3 to 1.0% in fresh leaves. After the leaves were dried, all populations maintained high EOs contents, which ranged from 0.5 to 2.7%. On the other hand, in winter, EOs contents ranged from 0.3 to 1.1% in fresh leaves and from 0.2 to 1.2% in dried leaves. Under these conditions, populations SF1, SF2, PI2, PE3, and NA1 showed higher contents (Table 2). We also observed an increase in EOs content in winter compared to summer in fresh leaves of populations BV2 and PE3 (Table 2).

Table 2. Essential oil content (%) of native populations of *Varronia curassavica* collected in the restinga of Santa Catarina, Brazil

Populations	Summer		Winter	
	Fresh	Dried	Fresh	Dried
SF1*	0.9 aA[#]	1.3 cA	1.1 aA	1.0 aA
SF2	0.7 aB	1.6 cA	0.8 aB	1.1 aB
BS1	0.7 aB	1.5 cA	0.4 bB	0.8 bB
BS2	0.4 bC	1.6 cA	0.6 bB	1.0 aB
BS3	0.9 aB	2.0 bA	0.9 aB	0.3 cC
BS4	0.5 bB	1.4 cA	0.5 bB	0.7 bB
BV1	0.6 bB	1.6 cA	0.8 aB	0.7 bB
BV2	0.6 bB	1.1 dA	1.1 aA	0.7 bB
BV3	0.3 bA	0.5 eA	0.5 bA	0.6 bA
PI1	0.8 aB	2.5 aA	0.3 bB	0.5 bB
PI2	0.7 aC	1.7 bA	1.1 aB	1.2 aB
PE1	0.7 aC	2.7 aA	0.4 bC	0.9 aB
PE2	0.3 bB	1.8 bA	0.7 aB	0.7 bB
PE3	0.6 bB	1.1 dA	0.9 aA	1.3 aA
NA1	1.0 aB	2.0 bA	1.0 aB	1.0 aB
NA2	0.6 bB	1.1 dA	0.8 aB	0.2 cC
Coefficient of Variation = 14.6 %				

Note. [#] Means followed by the same lower-case letter in the column and capital letter in the row do not differ statistically from each other by Scott Knott Test, at 5% probability level. * Populations: SF1, São Francisco do Sul; SF2, São Francisco do Sul; BS1, Balneário Barra do Sul; BS2, Balneário Barra do Sul; BS3, Balneário Barra do Sul; BS4, Balneário Barra do Sul; BV1, Barra Velha; BV2, Barra Velha; BV3, Barra Velha; PI1, Balneário Piçarras; PI2, Balneário Piçarras; PE1, Penha; PE2, Penha; PE3, Penha; NA1, Navegantes; NA2, Navegantes.

According to the EOs chemical profiles, 22 compounds were detected in summer and 18 in winter (Tables 3 and 4). We found that the chemical compounds with the highest relative proportion in summer were α-thujene (0.7-35.3%), α-pinene (4.1-45.6%), (*E*)-caryophyllene (1.5-25.4%), *allo*-aromadendrene (0.5-25.0%), α-zingiberene (0.8-44.4%), and β-sesquiphellandrene (0.8-23.0%) (Table 3). Other compounds, like (*E*)-nerolidol (20.9%) and α-muurolol (18.2%), were sampled in high amounts only in the fresh leaves of population PE1 in summer (Table 3). Moreover, α-thujene (2.1-38.5%), α-pinene (2.7-42.1%), (*E*)-caryophyllene (2.6-29.9%), *allo*-aromadendrene (0.9-45.4%), α-zingiberene (1.4-38.8%), β-sesquiphellandrene (1.3-22.0%), and spathulenol (1.0-20.6%) were present in the samples collected in the winter (Table 4).

The common chemical compounds found in all populations, regardless of season, were α-thujene, α-pinene, sabinene, α-humulene, (*E*)-caryophyllene, spathulenol, myrcene, allo-aromadendrene, β-sesquiphellandrene, and α-zingiberene (Tables 3 and 4). Drying influenced the less frequent substances in EOs. In all populations, γ-muurolene was not recorded in fresh leaves in summer but was detected in dried samples, except in populations PI1, PE1, and NA2 (Table 3). We also found that limonene was detected 77.7% more often in dried leaves in summer (Table 3). Furthermore, α-cubebene and β-copaen-4 α-ol were detected only in PI2 and BV1 populations, respectively, in dried leaves in winter (Table 4).

Table 3. Chemical composition of the essential oil of fresh and dried leaf samples from native populations of *V. curassavica* collected in the restinga of Santa Catarina, Brazil, during 2015/2016 summer

Chemical compounds	$^1RI^{cal}$ / $^2RI^{lit}$	Relative percentage of compounds by populations and drying (%)															
		SF1	SF2	BS1	BS2	BS3	BS4	BV1	BV2	BV3	PI1	PI2	PE1	PE2	PE3	NA1	NA2
α-thujene	925	12.4*	7.5	35.3	9.1	1.7	11.7	12.5	17.4	3.1	13.7	6.5	1.6	4.4	1.7	9.7	5.5
	924	6.0#	10.6	9.1	4.5	3.9	6.0	2.7	10.8	8.2	10.4	3.4	1.3	2.2	0.7	5.9	5.0
α-pinene	932	12.5	37.9	4.1	24.5	45.6	29.1	25.7	10.7	13.0	16.4	26.9	12.2	34.7	34.4	30.2	20.6
	932	17.0	34.6	12.8	29.6	15.6	25.0	7.7	24.9	19.9	10.0	19.9	11.3	26.3	42.5	19.1	20.2
sabinene	969	1.5	1.4	5.1	1.4	1.0	1.9	1.9	2.4	1.0	2.2	1.3	0.6	1.7	0.8	2.5	0.9
	969	0.7	1.5	1.7	1.1	0.7	1.2	0.4	2.5	1.2	1.6	0.7	0.8	1.0	0.6	1.3	1.0
β-pinene	974	-	-	-	0.5	4.2	1.6	4.0	-	5.7	-	3.5	1.4	7.6	2.5	4.5	-
	974	-	-	-	-	0.8	2.3	-	-	1.1	-	3.0	2.3	5.0	2.8	2.8	0.9
myrcene	989	0.5	0.5	0.9	0.4	0.9	0.6	0.7	0.5	0.7	0.5	0.7	0.4	1.5	0.7	0.9	1.0
	988	0.5	0.5	0.5	0.4	0.5	0.6	0.5	0.5	0.4	0.4	0.5	0.5	0.8	0.6	0.6	0.6
limonene	1028	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	0.8	-
	1024	4.0	2.4	0.2	1.1	3.6	0.4	-	-	-	-	-	0.7	-	-	1.4	1.6
Monoterpene hydrocarbons		**26.9**	**47.3**	**45.9**	**35.9**	**53.4**	**44.9**	**44.8**	**31.0**	**23.5**	**32.8**	**38.9**	**16.2**	**49.9**	**40.1**	**48.6**	**28.0**
		28.2	**49.6**	**24.3**	**36.7**	**25.1**	**35.5**	**11.3**	**38.7**	**30.8**	**22.4**	**27.5**	**16.9**	**35.3**	**47.2**	**31.1**	**29.3**
α-cubebene	1346	-	-	-	-	-	-	-	-	-	-	5.2	-	-	-	6.4	-
	1345	-	-	-	-	-	-	-	-	-	-	5.1	-	-	-	4.1	-
β-elemene	1387	-	1.9	3.0	1.5	1.6	2.3	0.5	-	-	-	1.0	-	3.2	1.5	1.0	-
	1389		3.0	1.1	2.6	0.4	1.7	-	-	2.3	0.4	-	-	0.3	0.7	0.7	-
(*E*)-caryophyllene	1416	4.4	5.3	4.8	9.1	6.5	6.1	3.5	1.5	3.0	7.6	7.9	8.5	8.4	8.5	5.7	6.1
	1419	9.8	8.4	21.6	19.9	6.7	13.2	2.9	8.3	15.3	20.9	19.9	4.1	25.4	18.3	7.6	10.7
α-trans-bergamotene	1433	1.7	0.5	-	0.6	-	0.6	0.7	1.4	2.0	1.6	-	-	-	-	-	1.8
	1432	1.1	0.9	0.2	0.7	1.4	0.7	1.8	0.7	0.4	1.1	0.4	1.7	-	-	0.5	1.2
α-humulene	1450	1.0	1.2	1.2	1.8	1.7	2.0	1.0	0.3	0.6	0.9	2.1	1.8	1.1	3.2	1.0	2.0
	1452	2.6	2.0	6.8	4.0	1.8	4.0	2.7	2.1	4.3	5.2	4.4	3.1	2.8	6.3	1.7	3.2
allo-aromadendrene	1458	1.3	14.0	22.7	15.5	21.1	10.8	17.0	11.0	0.6	0.7	10.8	15.8	19.2	12.4	10.8	1.0
	1458	1.3	20.5	25.0	14.4	2.6	7.2	0.5	19.4	17.0	1.8	10.2	0.5	18.1	15.4	4.6	1.2
γ-muurolene	1474	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1478	0.9	1.1	0.2	1.4	0.4	0.6	0.4	2.5	1.4	-	1.6	-	0.2	0.8	0.5	-
arcurcumene	1481	7.2	2.0	-	2.9	-	3.0	2.6	5.3	6.0	4.4	1.1	-	0.5	-	-	-
	1479	4.8	0.6	-	0.9	4.9	2.7	5.6	1.1	1.6	3.5	0.9	6.5	0.5	0.5	1.9	5.9
germacrene D	1489	-	1.0	1.2	1.0	1.7	0.8	0.8	1.0	0.8	1.7	2.2	2.8	1.3	1.0	1.3	9.4
	1484	1.6	-	0.3	1.9	-	1.0	-	5.1	3.1	-	2.3	-	-	1.6	-	-
α-zingiberene	1494	24.7	10.1	3.8	6.3	3.5	8.0	10.9	22.3	35.6	28.2	5.5	2.0	5.0	2.7	2.2	23.3
	1493	20.0	3.2	17.5	2.9	32.0	13.6	44.4	5.5	8.5	24.1	6.8	38.3	3.6	0.8	9.5	24.2
β-sesquiphellandrene	1520	12.5	4.9	1.1	4.3	0.8	4.7	5.5	10.8	15.8	12.4	3.3	2.1	2.1	2.1	2.0	13.3
	1521	12.3	8.3	1.1	2.4	17.9	7.8	23.0	13.8	5.3	13.0	3.8	20.8	11.1	1.2	7.1	15.5
Sesquiterpene hydrocarbons		**52.8**	**40.9**	**37.8**	**43.0**	**36.9**	**38.3**	**42.5**	**53.6**	**64.4**	**57.5**	**38.1**	**33.0**	**40.8**	**31.4**	**30.4**	**56.9**
		54.4	**48.0**	**73.8**	**51.1**	**68.1**	**52.5**	**81.3**	**58.5**	**59.2**	**70.0**	**55.4**	**75.0**	**62.0**	**45.6**	**38.2**	**61.9**
(*E*)-nerolidol	1562	0.5	-	-	-	-	-	-	-	0.5	0.4	-	20.9	-	-	-	-
	1561	-	-	-	-	-	-	-	-	1.6	-	-	-	-	-	-	-
spathulenol	1576	0.5	2.0	2.7	2.6	2.4	1.6	3.4	1.9	2.6	0.5	1.7	0.4	2.2	5.2	1.2	1.4
	1575	1.0	1.0	0.2	1.1	1.0	0.6	1.1	1.0	1.0	0.5	0.5	1.3	1.6	0.9	0.9	0.9
ar-turmerol	1580	0.8	1.2	-	-	-	1.2	-	-	0.4	-	-	-	-	-	-	1.5
	1582	0.9	-	1.2	1.1	0.5	1.1	2.0	1.0	1.0	1.2	0.8	-	0.3	1.3	0.9	0.6
ledol	1600	-	0.9	1.5	0.9	1.1	0.7	1.3	0.8	-	-	1.0	-	1.1	1.4	1.0	-
	1602	-	-	-	0.7	-	-	-	-	0.9	-	0.8	-	0.2	0.7	1.3	-
α-muurolol	1644	-	-	-	-	-	0.4	-	0.5	0.4	-	0.4	18.2	0.5	0.4	-	-
	1644	0.8	-	0.5	0.9	0.9	0.8	0.4	-	0.6	-	0.4	-	0.3	0.6	1.0	0.8
Oxygenated sesquiterpenes		**1.8**	**4.1**	**4.2**	**3.5**	**3.5**	**3.9**	**4.7**	**3.2**	**3.9**	**0.9**	**3.1**	**39.5**	**3.8**	**7.0**	**2.2**	**2.9**
		2.7	**1.0**	**1.9**	**38.0**	**2.4**	**2.5**	**3.5**	**2.0**	**5.1**	**1.7**	**2.5**	**1.3**	**2.4**	**3.5**	**4.1**	**2.3**
Total identified (%)		**81.5**	**92.3**	**87.9**	**82.4**	**93.8**	**87.1**	**92.0**	**87.8**	**91.8**	**91.2**	**80.1**	**88.7**	**94.5**	**78.5**	**81.2**	**87.8**
		85.3	**98.6**	**100.0**	**91.6**	**95.6**	**90.5**	**96.1**	**99.2**	**95.1**	**94.1**	**85.4**	**93.2**	**99.7**	**96.3**	**73.4**	**93.5**

Note. $^1RI^{cal}$ = Experimental Retention Index, $^2RI^{lit}$ = Literature Retention Index, Populations: SF1, São Francisco do Sul; SF2, São Francisco do Sul; BS1, Balneário Barra do Sul; BS2, Balneário Barra do Sul; BS3, Balneário Barra do Sul; BS4, Balneário Barra do Sul; BV1, Barra Velha; BV2, Barra Velha; BV3, Barra Velha; PI1, Balneário Piçarras; PI2, Balneário Piçarras; PE1, Penha; PE2, Penha; PE3, Penha; NA1, Navegantes; NA2; Navegantes; * Summer. # Winter. - content lower than 0.1%.

Table 4. Chemical composition of the essential oil of fresh and dried leaf samples from native populations of *V. curassavica* collected in tthe restinga of Santa Catarina, Brazil, during 2015/2016 winter

Chemical compounds	$^1RI^{cal}$ / $^2RI^{lit}$	Relative percentage of compounds by populations and drying (%)															
		SF1	SF2	BS1	BS2	BS3	BS4	BV1	BV2	BV3	PI1	PI2	PE1	PE2	PE3	NA1	NA2
α-thujene	925	9.6*	21.1	9.3	5.4	10.3	15.2	10.2	38.5	5.8	8.4	7.2	12.9	6.9	5.9	13.2	19.3
	924	5.8#	20.5	8.2	3.6	3.1	5.3	3.6	36.0	3.8	9.2	2.1	9.2	3.2	2.4	5.2	10.9
α-pinene	932	10.1	42.1	28.5	7.6	7.8	9.2	12.3	9.7	8.9	7.3	5.3	18.2	7.8	4.6	10.2	11.4
	932	14.6	31.0	16.8	5.1	15.6	15.3	8.9	7.6	10.2	9.4	7.8	13.3	9.2	2.7	8.3	9.7
sabinene	969	2.6	3.4	3.5	2.3	3.2	3.2	1.1	1.3	2.4	5.8	3.3	2.4	2.9	3.1	4.6	3.1
	969	5.2	2.8	8.6	4.3	1.4	2.7	2.0	2.9	1.8	8.4	3.5	2.0	4.5	2.5	6.5	2.5
β-pinene	974	2.2	-	-	-	-	4.1	3.6	-	3.4	-	5.6	3.2	9.8	-	2.5	-
	974	0.8	-	-	-	-	2.9	-	-	1.6	-	2.7	1.1	4.7	2.4	3.3	-
myrcene	989	4.3	1.0	3.8	2.6	2.0	1.0	1.6	1.2	1.7	4.8	2.1	1.0	9.5	2.7	1.2	4.2
	988	1.5	1.2	6.7	4.5	3.1	1.6	2.5	1.0	2.4	6.3	1.0	0.7	8.4	3.6	6.6	2.2
Monoterpene hydrocarbons		**28.8**	**67.6**	**45.1**	**17.9**	**23.3**	**32.7**	**28.8**	**50.7**	**22.2**	**26.3**	**23.5**	**37.7**	**36.9**	**16.3**	**31.7**	
		27.9	**55.5**	**40.3**	**17.5**	**23.2**	**27.8**	**17.0**	**47.5**	**19.8**	**33.3**	**17.1**	**26.3**	**30.0**	**13.6**	**29.9**	
α-cubebene	1346	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1345	-	-	-	-	-	-	-	-	-	-	8.4	-	-	-	-	-
β-elemene	1387	0.3	2.4	6.4	3.5	-	4.3	3.5	-	-	-	2.2	-	3.5	2.5	2.1	-
	1389	-	1.9	6.8	2.1	-	5.2	-	-	3.8	4.4	-	-	1.2	3.7	5.9	-
(*E*)-caryophyllene	1416	6.4	4.5	4.8	20.8	5.1	5.4	8.5	4.2	28.5	8.6	27.9	9.4	9.2	20.5	5.5	6.5
	1419	4.8	6.0	9.6	29.9	8.2	2.6	6.9	7.6	25.5	9.9	24.2	8.1	7.5	18.3	9.1	5.8
α-humulene	1450	1.0	0.8	1.3	1.2	1.2	2.1	1.0	0.5	1.1	0.3	1.1	1.6	1.3	2.0	2.8	1.2
	1452	1.4	1.0	2.5	1.1	1.5	2.2	1.2	5.0	2.7	3.2	2.1	1.8	1.2	3.1	2.4	1.0
allo-aromadendrene	1458	2.6	6.9	7.3	25.1	6.7	5.8	7.0	8.6	22.4	7.1	14.8	1.9	5.2	32.4	9.2	2.5
	1458	0.9	12.4	9.1	24.3	5.4	8.9	3.1	7.2	17.5	6.8	33.5	3.4	4.1	45.4	5.6	4.9
arcurcumene	1481	8.9	2.1	-	4.5	-	2.4	2.1	7.4	6.3	7.4	1.1	-	6.2	-	-	-
	1479	5.4	3.2	3.2	3.8	5.3	3.3	2.3	0.8	2.4	8.9	0.9	7.9	8.7	2.1	5.2	7.6
α-zingiberene	1494	23.5	5.6	4.6	5.2	25.5	17.6	13.7	6.5	7.4	8.4	3.1	16.2	4.3	2.5	13.5	23.2
	1493	32.4	1.4	5.1	3.6	20.4	12.1	38.8	7.6	8.9	7.6	2.5	25.7	5.4	1.6	10.5	20.2
β-sesquiphellandrene	1520	16.6	4.7	3.4	3.1	16.3	14.2	15.5	6.7	2.6	9.2	1.3	16.4	2.8	2.5	16.8	14.3
	1521	18.9	9.2	5.8	2.9	14.2	11.4	22.0	2.3	5.3	9.8	3.2	20.3	9.4	2.2	12.9	10.2
Sesquiterpene hydrocarbons		**59.3**	**27.0**	**27.8**	**63.4**	**54.8**	**51.8**	**51.3**	**33.9**	**68.3**	**41.0**	**51.5**	**45.5**	**32.5**	**62.4**	**49.9**	**47.7**
		63.8	**35.1**	**42.1**	**67.7**	**55.0**	**45.7**	**74.3**	**30.5**	**66.1**	**50.6**	**74.8**	**67.2**	**37.5**	**76.4**	**51.6**	**49.7**
spathulenol	1576	6.4	4.0	2.7	1.0	3.6	3.8	5.2	3.2	3.2	12.5	3.2	4.0	15.3	1.6	3.4	2.1
	1575	5.6	2.1	4.6	1.0	2.2	7.6	3.2	1.9	2.0	10.6	2.4	1.2	20.6	2.3	4.2	1.6
ar-turmerol	1580	1.0	-	3.8	-	-	2.1	-	-	1.4	-	-	-	-	-	-	1.0
	1582	-	-	5.6	2.4	1.0	1.9	-	1.3	1.1	3.4	-	-	0.3	1.1	2.7	4.9
ledol	1600	-	-	6.3	2.2	3.4	1.2	2.9	1.9	-	-	1.0	-	1.4	1.4	2.1	-
	1602	-	2.2	5.8	3.7	-	-	-	0.2	1.5	-	-	-	2.1	3.7	3.2	-
caryophyllene oxide	1575	-	-	3.4	2.6	-	4.3	-	3.2	2.6	6.5	2.3	4.2	-	2.5	4.2	3.2
	1582	-	1.9	-	5.0	-	6.5	-	-	-	-	-	-	-	-	-	-
copaen-4 α-ol	1592	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1590	-	-	-	-	-	-	5.1	-	-	-	-	-	-	-	-	-
Oxygenated sesquiterpenes		**7.4**	**4.0**	**16.2**	**5.8**	**7.0**	**11.4**	**8.1**	**8.3**	**7.2**	**19.0**	**6.5**	**8.2**	**16.7**	**5.5**	**9.7**	**6.3**
		6.6	**6.2**	**16.0**	**12.1**	**3.2**	**16.0**	**8.3**	**3.4**	**4.6**	**14.0**	**2.4**	**1.2**	**23.0**	**7.1**	**10.1**	**6.5**
Total identified (%)		**95.5**	**98.6**	**89.1**	**87.1**	**85.1**	**95.9**	**88.2**	**92.9**	**97.7**	**86.3**	**81.5**	**91.4**	**86.1**	**84.2**	**91.3**	**92.0**
		98.3	**96.8**	**98.4**	**97.3**	**81.4**	**89.5**	**99.6**	**81.4**	**90.5**	**95.9**	**94.3**	**94.7**	**90.5**	**97.1**	**91.6**	**81.5**

Note. $^1RI^{cal}$ = Experimental Retention Index, $^2RI^{lit}$ = Literature Retention Index, Populations: SF1, São Francisco do Sul; SF2, São Francisco do Sul; BS1, Balneário Barra do Sul; BS2, Balneário Barra do Sul; BS3, Balneário Barra do Sul; BS4, Balneário Barra do Sul; BV1, Barra Velha; BV2, Barra Velha; BV3, Barra Velha; PI1, Balneário Piçarras; PI2, Balneário Piçarras; PE1, Penha; PE2, Penha; PE3, Penha; NA1, Navegantes; NA2; Navegantes; * Summer. # Winter. - content lower than 0.1%.

We also found that α-humulene was detected in 100% of the samples (Tables 3 and 4), with contents ranging from 0.3 to 6.8% (Table 5). In the comparison between populations, PE3 obtained the highest EOs contents (2.0 to 6.8%), except in the fresh leaves of the BS4 population collected in winter (2.1%), where these populations were similar (Table 5). After the leaves were dried, all populations collected in summer showed high contents of α-humulene, varying from 1.7 to 6.8% (Table 5).

Table 5. Content of α-humulene (%) in the essential oil of native populations of *Varronia curassavica* collected in the restingas of Santa Catarina, Brazil

Populations	Summer		Winter	
	Fresh	Dried	Fresh	Dried
SF1*	1.0 gC[#]	2.6 hA	1.0 eC	1.4 gB
SF2	1.2 fB	2.0 jA	0.8 fD	1.0 iC
BS1	1.2 fD	6.3 bA	1.3 cC	2.5 cB
BS2	1.8 dB	4.0 eA	1.2 dC	1.1 hD
BS3	1.7 eB	1.8 lA	1.2 dD	1.5 gC
BS4	2.0 cD	4.0 eA	2.1 aC	2.2 dB
BV1	1.0 gC	2.7 gA	1.0 eC	1.2 hB
BV2	0.3 jD	2.1 iA	0.5 gC	1.0 iB
BV3	0.6 iD	4.3 dA	1.1 eC	2.7 bB
PI1	0.9 hC	5.2 cA	0.3 hD	2.2 dB
PI2	2.1 bB	4.4 dA	1.1 dD	2.1 eC
PE1	1.8 dB	3.1 fA	1.6 bC	1.8 fB
PE2	1.1 gD	2.8 gA	1.3 cB	1.2 hC
PE3	3.2 aB	6.8 aA	2.0 aD	3.1 aC
NA1	1.0 gB	1.7 mA	0.8 fC	0.6 jD
NA2	2.0 cB	3.2 fA	1.2 dC	1.0 iD
Coefficient of Variation = 2.94%				

Note. [#] Means followed by the same lower-case letter in the column and capital letter in the row do not differ statistically from each other by Scott Knott Test, at 5% probability level. * Populations: SF1, São Francisco do Sul; SF2, São Francisco do Sul; BS1, Balneário Barra do Sul; BS2, Balneário Barra do Sul; BS3, Balneário Barra do Sul; BS4, Balneário Barra do Sul; BV1, Barra Velha; BV2, Barra Velha; BV3, Barra Velha; PI1, Balneário Piçarras; PI2, Balneário Piçarras; PE1, Penha; PE2, Penha; PE3, Penha; NA1, Navegantes; NA2; Navegantes.

Chemical similarities between Eos extracted from populations harvested in summer and winter were investigated by multivariate analysis of the common chemical compounds, regardless of leaf drying. HCA using the Euclidean distance of 20 units as a measure of dissimilarity showed the formation of three clusters for the summer season (Fig. 1A). Cluster 1 comprised the populations SF1, PI1, NA2, BV2, and SF2, with α-thujene (5.0-17.4%), α-pinene (10.0-37.9%), (*E*)-caryophyllene (1.5-20.9%), *allo*-aromadendrene (0.7-20.5%), α-zingiberene (3.2-28.2%), and β-sesquiphellandrene (4.9-15.5%) as the main compounds. Cluster 2 encompassed the populations BV2, PE2, PE3, BS2, PI2, NA1, BS4, BV1, BS3, and P1, characterized by the main compounds α-thujene (0.7-17.4%), α-pinene (7.7-45.6%), (*E*)-caryophyllene (1.5-25.4%), *allo*-aromadendrene (0.5-21.1%), α-zingiberene (2.2-44.4%), and β-sesquiphellandrene (0.8-23.0%). Cluster 3 was represented by the population BS1, with α-thujene (35.3%), α-pinene (12.8%), (*E*)-caryophyllene (21.6%), *allo*-aromadendrene (25.0%), and α-zingiberene (17.5%) (Figure 1A).

In the winter season, we found eight clusters, using the Euclidean distance of 12 units as a dissimilarity measure (Figure 1B). Cluster 1 was formed by the populations SF1, NA2, BS3, BV1, and PE1, characterized by α-thujene (3.1-19.3%), α-pinene (7.8-18.2%), α-zingiberene (13.7-38.8%), and β-sesquiphellandrene (10.2-22.0%). Cluster 2 was composed of the BS4 and NA1 populations, with α-thujene (5.2-15.2%), α-pinene (8.3-15.3%), α-zingiberene (10.5-17.6%), and β-sesquiphellandrene (11.4-16.8%) among the major compounds. Cluster 3 comprised the populations BS2, BV3, PI2, and PE3, characterized by (*E*)-caryophyllene (18.3-29.9%) and *allo*-aromadendrene (14.8-45.4%). Finally, clusters 4, 5, 6, 7, and 8 were formed by one population each. In these populations, the main compounds were spathulenol (15.3-20.6%) in PE2 (Cluster 4); spathulenol (10.6-12.5%) in PI1 (Cluster 5); α-pinene (16.8-28.5%) in BS1 (Cluster 6); α-thujene (36.0-38.5%) in BV2 (Cluster 7); α-thujene (20.5-21.1%) and α-pinene (31.0-42.1%) in SF2 (Cluster 8) (Figure 1B).

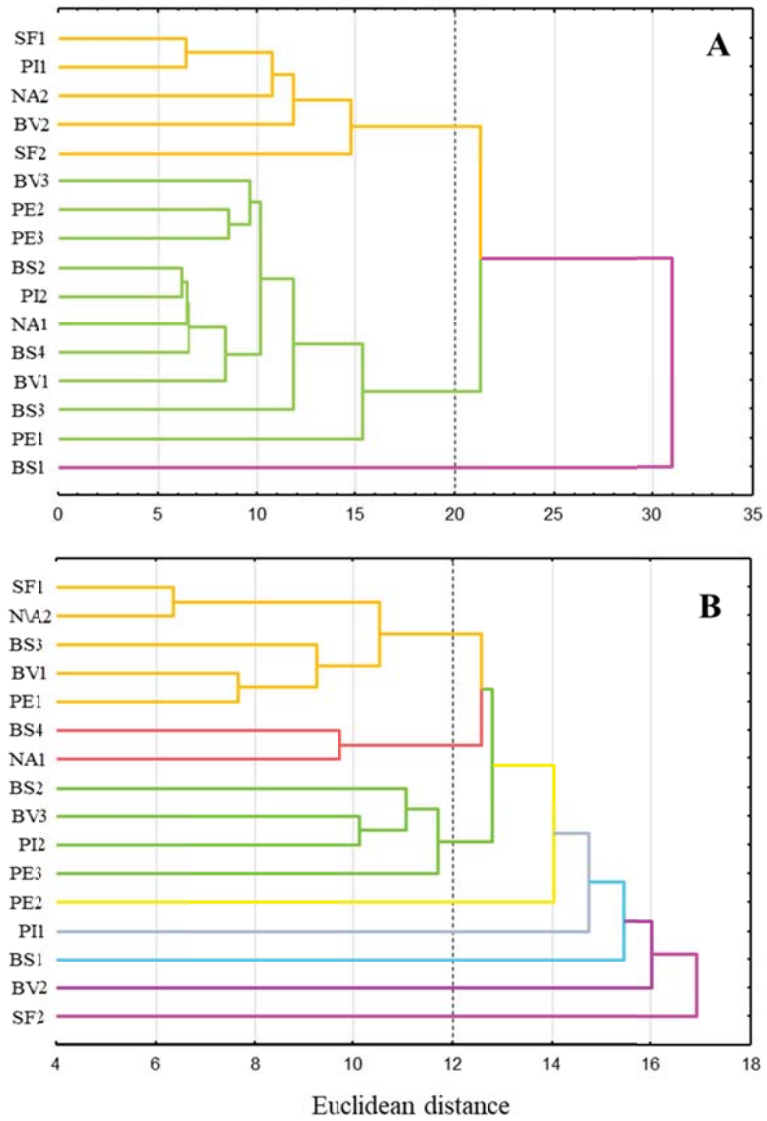

Figure 1. Hierarchical cluster analysis (HCA) of the common chemical compounds identified in the essential oils obtained from 16 native populations of *Varronia curassavica* collected in the restinga of Santa Catarina, Brazil, during in summer (A) and winter (B)

Note. A, summer; B, winter; Different colors in each figure represent the cluster formation. SF1, São Francisco do Sul; SF2, São Francisco do Sul; BS1, Balneário Barra do Sul; BS2, Balneário Barra do Sul; BS3, Balneário Barra do Sul; BS4, Balneário Barra do Sul; BV1, Barra Velha; BV2, Barra Velha; BV3, Barra Velha; PI1, Balneário Piçarras; PI2, Balneário Piçarras; PE1, Penha; PE2, Penha; PE3, Penha; NA1, Navegantes; NA2; Navegantes.

PCA was in line with the HCA results in most cases (Fig. 2). PCA analysis clustered the relationships between populations and the interrelationships between chemical compounds and soil and climate conditions those that showed similarities. In the summer season, the first principal component accounted for 41.5% of the total variation in the data, the compounds β-sesquiphellandrene, α-zingiberene, α-thujene, and α-pinene were associated to Cluster 1 and were inversely related to the compounds (*E*)-caryophyllene, α-humulene, and *allo*-aromadendrene associated to Cluster 2. According to PCA, the presence of such compounds in Cluster 2 is related to the soil and climate variables Tp, SB, OM, pH, V, and Pp. Cluster 1 and Cluster 3 are related to the P and Al variables (Figure 2A). The second principal component explained 23.1% of the total variation in the data, and α-pinene, myrcene, and spathulenol were related to the soils with lower P concentration (Figure 2A).

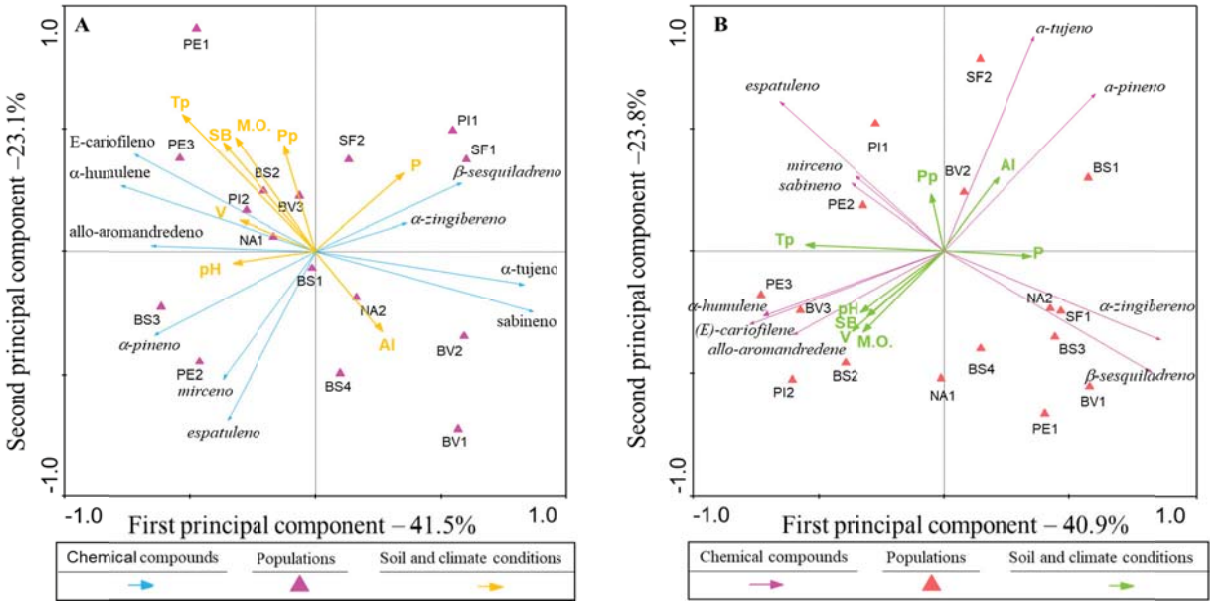

Figure 2. Principal Component Analysis (PCA) of the common chemical compounds identified in the essential oils obtained from 16 native populations of *Varronia curassavica* collected in the restinga of Santa Catarina, Brazil, during in summer (A) e winter (B)

Note. A, summer; B, winter; Tp, temperature; Pp, precipitation; Al, aluminum; OM, organic matter; P, phosphorus; pH, potential of hydrogen; SB, sum of bases; V, base saturation; SF1, São Francisco do Sul; SF2, São Francisco do Sul; BS1, Balneário Barra do Sul; BS2, Balneário Barra do Sul; BS3, Balneário Barra do Sul; BS4, Balneário Barra do Sul; BV1, Barra Velha; BV2, Barra Velha; BV3, Barra Velha; PI1, Balneário Piçarras; PI2, Balneário Piçarras; PE1, Penha; PE2, Penha; PE3, Penha; NA1, Navegantes; NA2; Navegantes.

For the winter season, Tp and P concentration were correlated with the first principal component, which explained 40.9% of the total variation in the data, with Tp influencing the increase in spathulenol and P increasing β-sesquiphellandrene and α-zingiberene (Figure 2 B). Populations PE3, BV3, BS2, and PI2 correlate positively with soil conditioning factors such as pH, OM, SB, and V, which are positively associated with (E)-caryophyllene, α-humulene and α-pinene, and negatively with α-thujene and sabinene, explaining 23.8% of the variance of the second principal component (Figure 2B).

4. Discussion

The EOs synthesized in the glandular trichomes of *V. curassavica* represent a mediation between plants and their surrounding environment. For the first time, our results showed that the EOs of this species collected from native populations of restinga in Santa Catarina, southern Brazil, exhibit different responses to soil and climate conditions. The SF1 and BV3 populations did not show variations in EOs content (0.9-1.3% and 0.3-0.6%, respectively). Although EO biosynthesis is determined by genetics, in the other populations studied, the variations in EO content suggest they have a geographical origin. Previous studies in other Brazilian states found that the EO content from *V. curassavica* populations ranged from 0.4% in São Paulo to 2.7% in Bahia, respectively (Marques et al., 2019). Queiroz et al. (2020) also showed differences in EO content (0.1-1.2%) in a germplasm bank of *V. curassavica* cultivated in Minas Gerais. On the other hand, analyses of populations of this species collected in San Rafael-Coxcatlán, Puebla, Mexico resulted in the lowest contents ever reported in the literature (0.1-0.3%) (Hernández et al., 2014). These comparisons suggest that some populations from Santa Catarina, such as PI1 (2.5%) and PE1 (2.7%), can be introduced as high EO content matrices for pharmaceutical industries. These responses in EO production benefit the selection of matrices used to determine growing conditions and improve productivity.

We observed that all populations increased EO content in the summer after the leaves were dried. This is due to the lower amount of water found in the dried samples that allows the vapor flow generated during hydrodistillation to drag the EOs stored in the plant tissues more efficiently. Evidence also indicates that the low water content in the dried plants decreases the tendency for the oil to bind, allowing the vapor to penetrate more uniformly into the tissues (Guenther, 1972). In this work, the flowering and fruiting period occurred almost

throughout the year, except in summer, when the plants were in the vegetative phase, as previously described (Brandão et al., 2015). This probably contributed to an increase in EO content in winter compared to the oil content extracted in summer in the fresh leaves of the BV2 (1.1%) and PE3 (0.9%) populations. The high rate of EO biosynthesis during the reproductive period in these populations may be due to the activation of enzymes required for the biosynthesis of certain compounds. The higher level of EO accumulated during flowering is a net result of the anabolic and catabolic processes during the several stages of flower development (Dubey & Luthra, 2001).

This research showed that the EOs chemical composition varied among the studied populations, highlighting the chemical diversity found in the same species. The common compounds in all populations were α-thujene, α-pinene, sabinene, α-humulene, (*E*)-caryophyllene, spathulenol, myrcene, *allo*-aromadendrene, β-sesquiphellandrene, and α-zingiberene, but with quantitative variations among them. A previous study on native populations of *V. curassavica* collected in Sergipe, Brazil, identified 53 compounds, of which 18 were also found in this study, except for α-thujene, myrcene, β-cubebene, γ-muurolene, (*E*)-nerolidol, and β-copaen-4 α-ol (Nizio et al., 2015). On the other hand, these compounds were obtained in *V. curassavica* genotypes cultivated in São Paulo, Brazil (Marques et al., 2019). These findings allow us to suggest that the diversity of compounds in the EO of *V. curassavica* may be influenced by geographic location compared to other studies (Santos et al., 2006; Nizio et al., 2015; Marques et al., 2019), as well as, the ability of the terpene synthase enzymes to convert the acyclic prenyl diphosphates and squalene into a multitude of cyclic and acyclic forms (Degenhardt et al., 2009). This property is found in almost half of the known monoterpenes and sesquiterpenes and may be attributed to the fact that the various reactive carbocationic intermediates can be stabilized in more than one way (Degenhardt et al., 2009). In this respect, we also observe that the chemical compounds' greatest diversity belongs to the classes of hydrocarbon sesquiterpenes and oxygenated sesquiterpenes compared to monoterpenes. Sesquiterpenes synthesized in the cytosol from farnesyl diphosphate by sesquiterpene synthases are structurally more diverse than monoterpenes due to the increased number of different cyclizations possible with five additional carbon atoms (Bohlmann et al., 1998; Nagegowda, 2010).

O α-humulene, an economically valuable constituent in the EO of *V. curassavica*, is a monocyclic sesquiterpene produced by terpene synthase enzymes using farnesyl pyrophosphate (Bohlmann et al., 1998). Its synthesis is related to the formation of CO_2 and acquisition of photosynthesis intermediates (Dehsheikh et al., 2019). Variations in the content of α-humulene (0.3-6.8%) found in this work may result from the influence of genetic load and environmental conditions. The α-humulene content was higher in the PE3 population (2.0-6.8%), regardless of season and leaf drying. A previous study found higher α-humulene content (31.6 %) in *V. curassavica* under field conditions with water and nutrition supply to the plants (Queiroz et al., 2020). In contrast, in our study, *V. curassavica* populations were established in sandy leached, and nutrient-poor soils, and plants were also exposed to high salinity, solar radiation, constant winds, and high soil temperatures. We also observed that all populations in summer, after the leaf drying process, maintained high α-humulene contents (0.7 to 6.8%). Also, we found that γ-muurolene, α-cubebene, and β-copaen-4 α-ol were detected only after the leaves were dried. Similarly, Amaral et al. (2017) found an increase in sesquiterpene molecules after leaf drying when studying tree species from the Atlantic Forest. These authors attributed that such changes in the EOs chemical composition are due to the higher stability of sesquiterpenes compared to monoterpenes and oxidation processes during drying.

We found intrapopulation variability of the chemical compounds in the EOs of *V. curassavica*. This variability plays an important role in understanding natural populations, as they outline conservation and genetic improvement strategies. Thus, we distributed the plants into groups regardless of leaf drying. As a result, HCA grouped the 16 populations into three groups in summer and eight in winter. PCA identified that (*E*)-caryophyllene, α-humulene, and allo-aromadendrene were strongly associated with populations PE1, PE3, BS2, PI2, BS4, and NA1 in summer. These populations were strongly related to climatic conditions in summer, such as Tp and Pp. The seasonal dynamics of the municipalities where the collections were made are quite similar, with hot and rainy summer (average values Tp of 24.5 °C; Pp of 240.1 mm) and mild and dry winter (average values Tp of 17.3 °C, Pp of 95.3 mm). However, with increased sunlight in summer, photosynthesis tends to increase, and consequently, high levels of energy are available for plant growth and development (Rezaei et al., 2019), resulting in the balance of available energy being directed toward secondary metabolite production. Similarly, the higher Tp recorded in summer provides considerable effects on substrate concentrations because of its effect on modifying day length (Burbott & Loomis, 1967).

The water availability is known to increase the production of terpenes (Maatallah et al., 2016) due to biosynthetic reactions occurring in an aqueous medium. Similar results were reported by Boira and Blanquer

(1998), who revealed a positive relationship of sesquiterpenes, such as β-caryophyllene and caryophyllene oxide, when Tp and Pp increased. On the other hand, we observed that Tp and Pp in winter are related to sabinene and α-thujene, although their relationships are less evident. These monoterpenes are inverse to (*E*)-caryophyllene, α-humulene, and allo-aromadendrene in winter. This negative relationship between monoterpenes and sesquiterpenes can be interpreted as competition between two pathways for the same precursor (Ghaffari et al., 2018; Ghaffari et al., 2019). Thus, we can suggest that isopentenyl pyrophosphate fluxes are dominant to the plastid (site of monoterpene synthesis) under winter conditions, whereas this flux tends toward the cytosol (site of sesquiterpene synthesis) in summer (McCaskill & Croteau, 1994).

Another factor considered in our work was the soil in which the plants grow. This is one of the main aspects that differentiated our research, contrasting with *V. curassavica* plants from different geographical origins and propagated under ideal growing conditions (Santos et al., 2006; Nizio et al., 2015; Marques et al., 2019). In this study, most of the collection sites belong to the order of arid soils. This order is typically defined by saline or alkaline soils with very little OM, characteristic of arid regions (Dewan & Famouri, 1964). Most of the collection sites belong to the order of arid soils. This order is typically defined by saline or alkaline soils with very little OM, characteristic of arid regions (Dewan & Famouri, 1964). As the habitats have distinct pedological characteristics, we identified that pH, OM, S, and V are associated with the synthesis of (*E*)-caryophyllene, α-humulene, and allo-aromadendrene from populations PE3, BV3, BS4, PI2, and BS2, acting inversely with Al in the soil. The positive effects of these chemical compounds associated with pH affect plant nutrient availability and natural soil fertility (OM, S, and V). On the other hand, by acting inversely with Al, they decrease the toxic effects of this element on plants. Al impairs the synthesis of energy in the plant due to the inhibition of P uptake and transport and the ATPase enzyme activity (Ahn et al., 2001; Abichequer et al., 2003). Considering the need for energy in the form of ATP and other P-dependent enzymes in the synthesis of EOs (Loomis & Corteau, 1972), the limitation of this element may be determinant in the increase of (*E*)-caryophyllene, α-humulene and allo-aromadendrene contents under conditions of Al saturation in the soil. Thus, the chemical variation of EOs observed in *V. curassavica* populations is due to environmental conditions, as well as their interactions.

5. Conclusion

The PI1 and PE1 populations exhibit the highest EO content, while PE3 has the highest α-hulene content regardless of drought and season. HCA demonstrates differences in the chemical profile of populations from different locations. PCA corroborates these findings and shows that (*E*)-caryophyllene, α-humulene and allo-aromadendreno are related to soil and climate conditions (Tp, Pp, OM, SB, V and S) of PE1, PE3, BS2, Populations PI2, BS4 and NA1 in summer.

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Effects of the Adoption of Technology Combinations Beyond Standardized Systems on the Income of Chinese Tobacco Farmers

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Abstract

Using the microdata for tobacco farmer households in Chongqing, China, this article analyses the determinants of adopting additional multiple agricultural technologies and their impact on income based on implementing a standardized technology system by a tobacco company. In this paper, selection bias from the observed and unobserved heterogeneity was corrected using a multinomial endogenous treatment effects model, and the endogenous properties were eliminated. The empirical results show that the adoption of a variety of additional agricultural technologies was determined by famer's education level, years of tobacco planting, household size, number of technical training sessions, distance from farmer's family to the nearest tobacco technology extension station, distance from farmer's family to the nearest township, proportion of land suitable for machine farming, proportion of leased land. Different from empirical judgment, integrated pest management and balanced fertilization are the most effective additional technology combination strategies for increasing farmers' income instead of combining all additional comprehensive technologies. The research results suggest that Chongqing Tobacco Company should further strengthen the training of tobacco farmers and guide tobacco farmers to take appropriate pesticide and fertilizer input beyond the standardized technical system, especially for those tobacco farmers far away from the tobacco technology extension station.

Keywords: tobacco, income, technology, joint adoption, Chongqing, China

1. Introduction

In the process of agricultural production, farmers often face a series of technical choices that can be adopted individually or jointly (Feder, Just, & Zilberman, 1985; Mutale, Kalinda, & Kuntashula, 2017; Tesfaye, Blalock, & Tirivayi, 2020). Since Feder (1982) first developed a model to deal with interrelations in the adoption of multiple agricultural technologies (MATs), an increasing number of researchers have studied a variety of technology combination schemes (Cafer & Rikoon, 2018; Khanna, 2001; Kimbi et al., 2021; Wu & Babcock, 1998).

Most previous studies concluded that the more diversified the technology combination adopted by farmers, the better. The typical research evidence is that combining new varieties with complementary agronomic techniques and resource management practices can result in higher yields and income (Kassie et al., 2018; Tufa et al., 2019). Beaman, Karlan, Thuysbaert, and Udry (2013) found that with the increase in fertilizer use, female rice farmers in Mali optimized modern agricultural inputs, such as herbicides, obtaining a combined effect. Emerick, de Janvry, Sadoulet, and Dar (2016) conducted a randomized experiment in India. They found that a new rice variety and introducing other technologies (*e.g.*, fertilizer and labor-intensive cropping methods) can increase income through factor deepening effects, downside risk effects, and wealth effects. Kassie et al. (2018) found that farmers in Ethiopia who adopted improved maize seeds, chemical fertilizers and legume diversification generated the highest payoffs. Khonje, Manda, Mkandawire, Tufa, and Alene (2018) showed that compared to farmers who adopted one measure alone, farmers in eastern Zambia who adopted Climate-smart agriculture (CSA) interventions using both conservation agricultural practices (CAPs) and improved maize varieties (IMVs) obtained maximum yields and

household income. Martey, Etwire, Mastenbroek, and Abdoulaye (2020) found that farmers in Ghana increased their income by adopting a combination of two CSA technologies: row planting and drought-tolerant maize seeds. In a study of Kenyan mango farmers, Midingoyi, Kassie, Muriithi, Diiro, and Ekesi (2019) highlighted that transitioning from one type of integrated pest management (IPM) to multiple IPM practices generated more economical, environmental and human health benefits. Tambo and Mockshell (2018) showed that the joint adoption of three components of conservation agriculture (CA) by farmers in Sub-Saharan Africa (SSA) had the greatest benefits. Teklewold, Kassie, Shiferaw, and Köhlin (2013) found that Ethiopian farmers who adopted a combination of three sustainable agricultural practices (SAPs), *i.e.*, cultivation system diversification, conservation tillage and modern maize seeds, generated the highest income. A study by Zeweld, Van Huylenbroeck, Tesfay, Azadi, and Speelman (2020) in Ethiopia indicated that the combination of three sustainable development technologies (contour terracing or water and soil conservation, animal manure and crop residues) resulted in the highest per capita income.

However, some scholars have proposed different opinions, suggesting that technology combination schemes that are more diverse are not always better. Adolwa, Schwarze, and Buerkert (2019) found that farmers in northern Ghana and western Kenya adopted integrated soil fertility management (ISFM) to increase maize production. However, increasing the number of ISFM components did not improve maize yield or total household income. Ainembabazi et al. (2018) reported that farmers in Central Africa who adopted either one technical package or a combination of two technical packages (but not all), *i.e.*, AR4D technologies (improved crop varieties (IVs)), crops and natural resource management (CNRM), and post-harvest (PH) technology, gained much greater benefits related to reducing poverty than those who adopted all technologies simultaneously. Di Falco and Veronesi (2013) found that in the Nile basin of Ethiopia, farmers who simultaneously adopted three climate change adaptation strategies, including water conservation strategies, soil conservation strategies, and crop rotation, did not generate a higher net income than those who adopted two strategies. Manda, Alene, Gardebroek, Kassie, and Tembo (2016) found that the combined use of SAPs in Zambia was more favorable to increasing income than SAPs alone. However, the per capita income was not the highest with the simultaneous adoption of three SAPs. Mutenje, Kankwamba, Mangisonib, and Kassie (2016) found that farmers in Malawi who adopted three technologies, *i.e.*, improved maize, soil and water conservation, and improved storage, simultaneously increased per hectare maize yield by 10% compared to 14% for those who adopted improved maize alone and 29% for those who adopted improved storage and improved maize.

The different conclusions of the above studies indicate that different technologies are not mutually independent (Kassie, Jaleta, Shiferaw, Mmbando, & Mekuria, 2013; Teklewold et al., 2013) and that a complementary, substitute or supplementary function is commonly present between technologies in technical combination schemes (Kassie, Teklewold, Jaleta, Marenya, & Erenstein, 2015a). Amadu, McNamara, and Miller (2020), and Kassie, Teklewold, Marenya, Jaleta, and Erenstein (2015b) also showed that synergies among technical combinations could promote income growth. Zeweld et al. (2020) reported that the combination of animal manure and retained crop residue resulted in a negative yield effect, likely due to the substitution effect between the two and simultaneous use resulting in a decrease in yield.

Existing studies mainly focused on rice， maize and wheat (Mishra, Khanal, & Pede, 2017; Mastenbroek, Sirutyte, & Sparrow, 2021; Abate, Bernard, Brauw, & Minot, 2018). This study takes tobacco as the research object. Tobacco is an important economic crop in developing countries and is generally produced based on contracts (including in China) (Appau, Drope, Witoelar, Chavez, & Lencucha, 2019; Appau et al., 2020; Briones, 2015; Magati, Lencucha, Li, Drope, & Zulu, 2019; Makoka et al., 2017; Scoones, Mavedzenge, Murimbarimba, & Sukume, 2018). Every year, Chongqing Tobacco Company (CTC) develops a set of standardized technical systems as a compulsory production standard. Based on our long-term field observations, and due to land heterogeneity and complex climate changes, tobacco farmers commonly adopt MATs in addition to strictly implementing standardized technical systems to maximize their income. These additional technologies mainly include IPM, balanced fertilization (BF), and soil improvement (SI). This paper is different from previous studies. First, we focus on tobacco as a special agricultural product. It also significantly contributes to households' income in the study area. The Framework Convention on Tobacco Control (FCTC) advocated a gradual reduction in tobacco supplies worldwide. China has a national tobacco monopoly with a strict quota management system. There are very few studies on the adoption of tobacco technology. Dimara and skuras (1998) studied the adoption of a new flue-cured tobacco variety in Greece. Omara, Odongo, and Kule (2021) assessed tobacco farmers' perception and factors affecting the adoption of rocket barn technology in Uganda. Second, this paper studies the income effect of multiple technical combinations beyond the scope of standardized technology systems. Previous studies have mainly focused on standardized technologies without considering land heterogeneity and the individuality of the

farmers. Third, this study considers the quality of heterogeneity of tobacco. Previous studies generally assumed that agricultural products were homogenous and seldom took into account that prices increased as the quality grade increased. Before the production season, the China National Tobacco Company (CNTC) releases price rankings for 40 grades of tobacco. The prices of different quality grades vary greatly, significantly affecting the income of tobacco farmers. The findings of this study will contribute to help tobacco farmers achieve income growth within the limits of the tobacco production quota for the FCTC.

2. Tobacco-Planting Technology in Chongqing

Tobacco is an important economic crop in China, and tobacco planting area and yields rank first in the world. Chongqing's tobacco planting distribution encompasses 12 districts and counties in the Three Gorges Reservoir Area and the Wuling Mountainous Area, which lies one of the major tobacco-producing region in China. The average elevation of the area is about 800-1400 m, typical for tobacco-growing regions in the mountains. Tobacco is one of the few economic crops in the areas due to the natural environmental factors which are similar to tobacco-producing areas in Indonesia and the Philippines (Appau et al., 2019). In 2018, approximately 405,700 mu (1 mu = 1/15 hectares) of tobacco were planted, and 46.7 million kg of flue-cured tobacco were produced.

At the beginning of each year, CTC publishes the purchase price per unit of each grade of tobacco and, at the same time, signs cultivation contracts with tobacco farmers, agreeing on planting area, unit yield and total output based on the production quota. CTC sets up tobacco technology extension stations in various planting areas so that tobacco technicians can train, guide and supervise tobacco planting following the standardized technical system. Additionally, they organize tobacco farmers into cooperatives (farmers are all members of cooperatives) and help solve technological difficulties in the planting process.

In December 2008, CTC applied the ISO9000 quality standard management system for tobacco planting. Each year, a technical programme for planting under normal weather conditions is developed in advance to provide a standardized technical system, as mandatory production specifications cover the entire tobacco planting process. CTC provides a unified and specialized service for some processes that are labor intensive and have high technical requirements, such as raising seedlings, mechanized farming, plant protection, flue-curing, and grading. Each household performs transplanting, uncovering, weeding, topping and pruning, and picking separately. Due to differences in climate, elevation, and vegetation and differences in soil thickness, fertility, and slope in tobacco-growing areas, tobacco farmers generally adopt multiple additional agricultural technologies. Chongqing tobacco farmers usually adopt three main additional technologies: IPM, BF and SI.

3. Methodology

The present study is based on diffusion of innovations theory (Rogers, 2003) and induced innovation theory (Binswanger & Ruttan, 1978; Ruttan & Hayami, 1984). The diffusion of innovations theory held that the characteristics of innovation, such as comparative advantage and testability, would determine its adoption rate. Farmers usually consider the potential benefits, which include increased production, reduced cost, enhanced efficiency and so on, when deciding on new technologies. The induced innovation theory emphasized that farmers were influenced and induced by the change of factor price, and would strive to adopt the technology that can replace the scarce resources, thus the relative abundance of resource endowment would affect the adoption of technology, such as labor and capital. Adoption decisions on agricultural technology depend on the individual optimization of expected utility or earnings (Feder et al., 1985). Michler, Tjernström, Verkaart, and Mausch (2019) argued that relative to yield targets, farmers focus more on economic return indicators when making decisions. Based on Khonje et al. (2018) and Tesfaye et al. (2020), this study used a random utility framework to analyse the adoption of multiple additional agricultural technologies, including IPM, BF, and SI, in a total of seven possible combinations: (i) IPM only, (ii) BF only, (iii) SI only, (iv) IPM & BF, (v) IPM & SI, (vi) BF & SI, and (vii) IPM & BF & SI. It is assumed that tobacco farmers aim to maximize their utility by comparing various combinations of technology combination options. Therefore, if $V_{ij} > V_{ik}$, $k \neq j$, tobacco farmer i selects technology combination j instead of any other combination k.

When making decisions regarding adopting technology, tobacco farmers are affected by many factors, including observable and unobservable factors, which may lead to endogeneity problems. For example, tobacco farmers may decide to adopt a technology combination scheme based on their innate management and technical capabilities related to understanding and using technology (Abdulai & Huffman, 2014). If we do not consider unobservable factors, the real impact of MATs may be overestimated or underestimated.

With the empirical approach of Manda et al. (2016) as a reference, a multinomial endogenous treatment effects model (Deb & Trivedi, 2006b) is used herein to explain the selection bias due to the observed and unobserved heterogeneity and to evaluate the differential effects of multiple agricultural technology combinations. When

assessing adoption decisions, the multinomial endogenous treatment effects model allows the modelling of interdependence among different technologies. Compared with the multinomial endogenous switching regression (MESR) method adopted by many researchers (Di Falco & Veronesi, 2013; Martey et al., 2020), the multinomial endogenous treatment effects model is easier to implement and allows the distribution of endogenous treatments (adoption of MATs) and output outcomes (such as yield and income) to be specified using a latent factor structure, thereby distinguishing unobservable and observable choices (Deb & Trivedi, 2006b). The multinomial endogenous treatment effects model improves the estimation effect by reducing the endogenous impact.

This model not only considers the interdependence of adoption decisions but also considers selection bias due to observed and unobserved characteristics. In the first stage, a mixed multinomial logit selection model was used to model adoption decisions. In the second stage, ordinary least squares (OLS) and selectivity correction were used to estimate the effects of the adoption of additional MATs on yield per mu (YPM), the average sales price of tobacco (ASPT), and income per household labor engaged in tobacco planting (IPHL). It is hereby noted that ASPT is equal to total tobacco revenue by total output, which reflects the level of tobacco quality grade.

3.1 Multinomial Endogenous Treatment Effects Model

The multinomial endogenous treatment effects model has two stages. In the first stage, the tobacco farmers selected one of the above seven MAT combinations. Based on Deb and Trivedi (2006a, 2006b), V_{ij}^{*} indicates the indirect utility associated with the jth MAT combination, $j = 0, 1, 2...$, and i represents tobacco farmers:

$$V_{\mathrm{ij}}^{*} = z_i^{'}\alpha_j + \sum_{k=1}^{J}\delta_{jk}l_{ik} + n_{ij} \tag{1}$$

where, z_i is the vector of the covariates, such as characteristics of tobacco farmers and plot features discussed in Section 3.3; α_j is the vector of the corresponding parameter to be estimated; n_{ij} is an independent and identically distributed error term; and l_{ik} is a latent factor that includes the unobserved farmer and tobacco field characteristics shared by the tobacco farmers in the adoption of MATs and their output outcomes as well as the influence of observable factors that may be associated with the outcome variables (Abdulai & Huffman, 2014; Pannell, Llewellyn, & Corbeels, 2014). In the above equation, $j = 0$ means non-adopter, and at this point, $V_{i0}^{*} = 0$. Although V_{ij}^{*} is not observed, the choice of MAT combinations is a set of binary variables d_j. The set of these variables can be expressed as vectors $d_i = d_{i1}, d_{i2}, \ldots d_{iJ}$. Similarly, let $l_i = l_{i1}, l_{i2}, \ldots l_{iJ}$; then, the probability of processing can be written as follows:

$$\Pr\left(d_i|z_i,\ l_i\right) = g\left(z_i^{'}\alpha_1 + \sum_{k=1}^{J}\delta_{1k}l_{ik} + z_i^{'}\alpha_2 + \sum_{k=1}^{J}\delta_{2k}l_{ik} + \cdots + z_i^{'}\alpha_J + \sum_{k=1}^{J}\delta_{Jk}l_{ik}\right) \tag{2}$$

where, g is the appropriate distribution of multinomial probability. If g has a mixed multinomial logit (MMNL) structure, g is defined as follows:

$$\Pr(d_i|z_i,\ l_i) = \frac{\exp\left(z_i^{'}\alpha_j + \delta_j l_{ij}\right)}{1+\sum_{k=1}^{J}\exp(z_i^{'}\alpha_k + \delta_k l_{ik})} \tag{3}$$

In the second stage, we assessed the effect of adopting the MAT combinations on the three outcome variables, namely, the natural logarithms of YPM, ASPT, and IPHL. The formula is as follows:

$$E(y_i|d_i,\ x_i,\ l_i) = x_i^{'}\beta + \sum_{j=1}^{J}r_j d_{ij} + \sum_{j=1}^{J}\lambda_j l_{ij} \tag{4}$$

In this equation, y_i is the output outcomes of tobacco farmer i, x_i represents the exogenous covariate for parameter vector β, and parameter r_j represents the effect of MATs on the output outcome of tobacco farmers. The variable d_{ij} is the coefficient parameter of r_j; if d_{ij} is assumed to be exogenous and the decision to adopt MATs is endogenous, the estimation of r_j would be inconsistent. The latent factor is l_{ij}, indicating that the unobserved characteristic variables can affect not only the output outcomes but also the selection of technology combination schemes. The load factor λ_j represents the direction of the correlation between the treatment effect and the outcome variable. When λ_j is positive (negative), the treatment effect and outcome variable are positively (negative) related through unobserved characteristics, *i.e.*, positive (negative) choice exists. Because the outcome variables are continuous, it is assumed that they follow a normal (Gaussian) distribution function. We estimated the model using the maximum simulated likelihood (MSL) method.

The instrumental variable approach is generally adopted to solve the endogeneity of technology adoption decisions (Suri, 2011). The selected instrumental variables will impact the adoption of technological schemes but will not directly affect the output outcomes (such as yield and income) except through the adopted decisions. Based on Gao, Niu, Yang, and Yu (2019), Manda et al. (2016), and Khonje et al. (2018), the instrumental variables selected in this paper included the distance from farmer family to tobacco technology extension stations (This variable reflects

how convenient it is for tobacco farmers to obtain technical information and technical guidance from tobacco technicians), the proportion of flat land area (the ratio of land suitable for mechanized farming to the total tobacco farmland), and the proportion of leasehold land (the ratio of leasehold land to the total tobacco farmland). We show that these three instrumental variables are effective through a simple falsification test and that they jointly affect MATs adoption decisions (test results in Table 3) but do not affect the output outcome variables (test results in Table A3).

3.2 Data Collection

Cross-sectional survey data for tobacco farmers in Chongqing were used in this study. The survey was conducted in all 12 tobacco-planting districts/counties in Chongqing from March to June 2019, and a total of 500 questionnaires were used to collect data. The sample size for the questionnaire survey in each district/county was determined based on the tobacco production quota, and trained professionals randomly selected tobacco farming villages to conduct household surveys, distribution of questionnaires is shown Table A1. Before the official start of the survey, a pre-survey was conducted in Qianjiang District, one of the main production areas, in February of the same year to supplement and improve the questionnaire design and investigation process.

The questionnaire collected comprehensive information on heads of household and families, knowledge of tobacco-planting technology, and technology adoption in 2018, specifically including the adoption level of the standardized technology system promoted by CTC and the adoption of additional technologies, *i.e.*, IPM, BF, and SI. We also obtained the actual data for tobacco farmers' acquisition information for 2018 through the Chongqing Tobacco Science Research Institute; these data included sales volume, quality grade, average sales price, and sales revenue for each tobacco farmer.

We defined the additional three technologies as binary variables. If the farmers chose to purchase and use pesticides on at least one plot following the list of pesticides recommended by CTC, then IPM took a value of 1; otherwise, it took a value of 0. If the farmers chose to purchase and use fertilizers on at least one plot in accordance with the list of fertilizers recommended by CTC, then BF was set to 1; otherwise, it was set to 0. SI was set as 1 if the tobacco farmer used measures such as deep ploughing, green manure planting and tilling, and the application of dolomite powder, oyster shell powder or lime to adjust the pH value on at least one plot; otherwise, it was set to 0.

This paper adopted a similar strategy as Suri (2011). It did not include household labor in tobacco farming costs, and the return function was approximated with a total income function for technology adoption. Therefore, the survey did not collect specific data on the planting costs of tobacco farmers.

A total of 384 valid questionnaires were finalized, for an effective rate of 76.80%. The low effective rate was a result of matching the information from the questionnaire with tobacco farmers' acquisition information one by one and excluding the missing data and tobacco farmers who did not grow tobacco in 2018 to ensure objective and realistic results.

3.3 Description of the Variables

Based on theoretical analyses and combing the previous literature (Di Falco, Bezabih, & Yesuf, 2010; Feder et al., 1985; Lee, 2005), this study used indicators such as head of household characteristics (gender, age, education level, and years of tobacco farming), household characteristics (family size and tobacco farming labor), and plot characteristics (tobacco farming area); furthermore, the following variables related to the actual situation of tobacco farmers in Chongqing were considered.

Labor force ratio: This variable reflects the ratio of the labor force (16-65 years old) to total family size.

Number of training sessions: Taking into account that tobacco farmers have regular access to technical training from CTC and technical guidance from tobacco technicians, the number of times that tobacco farmers participated in agricultural technology training in a year was selected to reflect farmers' knowledge and mastery of various planting technologies.

Loan: Farmers can easily obtain credit support from banks with a tobacco company's planting contract. Most tobacco farmers' households generally have good cash flow income, and their own funds can guarantee planting needs. Therefore, the collected information only reflects whether the farmers have taken a loan from a bank.

Distance to the nearest township: The distance to the nearest township reflects the transaction costs of planting inputs, hiring workers, and the availability of new technology, information and credit institutions (Kassie et al., 2013).

4. Results and Discussion

4.1 Descriptive Statistics

Table 1 provides the descriptive statistics of relevant variables. Considering that only one sample did not adopt any of the three technologies, this sample was excluded, and 383 samples were used for the empirical analysis. On this basis, we generated new combinations whose distribution is shown in Table 2.

Table 1. Descriptive statistics of the variables

Variables	Description	Mean	SD
Outcome variables			
YPM	Yield per mu (kg/mu)	114.01	30.06
ASPT	Average sales price (Yuan[a]/kg)	27.77	2.13
IPHL	Income per household labour engaged in tobacco planting (ten thousand RMB)	6.00	3.96
Treatment variables			
IPM	Adopt IPM technology in at least one plot (1 = yes)	0.79	0.41
BF	Adopt BF technology in at least one plot (1 = yes)	0.95	0.22
SI	Adopt SI technology in at least one plot (1 = yes)	0.87	0.33
Explanatory variables			
Age	Age of the head of household (years)	49.97	7.10
Gender	Gender of the head of household (1 = male)	0.93	0.25
Education [b]	Formal education experience of the head of household	2.65	0.73
Planting years	Years of tobacco farming (year)	20.55	9.93
Household size	Family size (number of people)	4.89	1.43
Social capital	Village cadre in the family (1 = Yes)	0.15	0.36
Planter	Tobacco farming labour (number of people)	2.08	0.63
Area	Area of tobacco farming (mu)	38.16	22.73
Distance1	Distance to the nearest township (km)	8.59	7.08
Loan	Loan (1 = Yes)	0.43	0.50
Train	Number of technical training sessions during the year	5.20	2.51
Labour force ratio	Ratio of labour force (16-65 years old) to total family size	0.69	0.23
Instrumental variables			
Distance2	Distance to the nearest tobacco technology extension station (km)	5.22	4.81
Flatland	Proportion of land suitable for machine farming to area of total tobacco farmland	0.72	0.30
Leaseland	Proportion of leasehold land to the area of total tobacco farmland	0.67	0.51
Number of obs.		384	

Note. [a] Yuan is the Chinese currency: 1 USD = 6.62 Yuan in 2018. [b] 1, 2, 3, 4, and 5 indicate never attended school, elementary school, junior high school, high school (secondary), or junior college or above, respectively.

Table 2. Proportion of tobacco farmers who adopted different technical combinations

	IPM only	BF only	IPM & BF	SI only	IPM & SI	BF & SI	IPM & BF & SI	Total
Freq.	4	4	40	6	8	70	251	383
Percent	1.04	1.04	10.44	1.57	2.09	18.28	65.54	100.00

Note. From the sample structure analysis, the sample sizes for IPM only, BF only, SI only and IPM & SI are small and not representative. Therefore, this paper focuses on three combinations: IPM & BF, BF & SI, and IPM & BF & SI. The descriptive statistics of these technology combinations are shown in Table A2.

Table 3. Estimate of the mixed multinomial logit model using MATs in Chongqing, China (baseline category is the adoption of the additional three MATs)

Variables	IPM only	BF only	IPM & BF	SI only	IPM & SI	BF & SI
Age	-0.0804 (0.115)	0.0571 (0.0953)	0.0277 (0.0282)	0.0446 (0.0807)	0.0273 (0.0600)	0.00461 (0.0252)
Education	-0.788 (1.180)	0.603 (1.158)	0.000408 (0.259)	-1.939** (0.852)	-0.231 (0.630)	-0.623** (0.247)
Years	0.0315 (0.0777)	-0.175* (0.101)	-0.00485 (0.0202)	-0.0471 (0.0582)	0.0374 (0.0514)	-0.0799*** (0.0187)
Homesize	-1.630* (0.844)	2.072* (1.156)	0.272* (0.153)	0.102 (0.408)	-0.291 (0.304)	-0.0349 (0.149)
Planter	1.575 (1.082)	0.0201 (0.936)	0.217 (0.280)	0.274 (0.945)	-0.641 (0.831)	0.286 (0.258)
Area	0.0179 (0.0256)	0.00839 (0.0487)	0.0197** (0.00841)	0.0408* (0.0225)	0.00920 (0.0255)	0.0236*** (0.00808)
Distance1	-0.124 (0.126)	0.213* (0.109)	0.0681*** (0.0241)	-0.109 (0.135)	-0.0724 (0.0812)	-0.0541 (0.0337)
Loan	-0.185 (1.369)	-0.635 (1.777)	-0.339 (0.393)	0.0154 (1.062)	-0.779 (0.995)	-0.442 (0.355)
Train	-0.0163 (0.220)	0.0921 (0.455)	0.105 (0.0724)	0.358* (0.198)	-0.351* (0.200)	0.230*** (0.0664)
Labour	-3.503 (2.181)	14.54 (9.533)	0.192 (0.981)	-2.048 (2.539)	-2.680* (1.628)	-0.114 (0.868)
Instrumental variable						
Distance2	0.119 (0.120)	-0.229 (0.298)	-0.0608 (0.0398)	-0.398* (0.218)	-0.217 (0.180)	-0.261*** (0.0611)
Flatland	-3.922* (2.261)	1.728 (4.167)	-1.111* (0.609)	-2.132 (1.791)	-2.034 (1.266)	-1.761*** (0.568)
Leaseland	0.171 (1.305)	-6.953** (3.326)	-0.314 (0.638)	0.283 (0.689)	0.339 (0.492)	-2.129*** (0.547)
Constant	8.653 (8.820)	-28.89** (14.55)	-5.628*** (2.022)	-0.803 (6.183)	3.285 (4.312)	3.288* (1.943)
Joint significance of instrumental variables $\chi^2(18) = 47.87^{***}$						
Wald test $\chi^2 = 114.97$; $P > \chi^2 = 0.0041$						

Note. *** $p < 0.01$, ** $p < 0.05$, * $p < 0.1$. Standard errors in parentheses.

4.2 Determinants of MATs Adoption

It was noted that 78.91% of tobacco farmers adopted IPM, 95.05% adopted BF, and 87.24% adopted SI, while more than 65% of tobacco farmers adopted a combination of the three types of technology. Considering the nature of the logit model, we used adoption of the three additional technologies as the baseline. This approach is different from those taken by Manda et al. (2016) and Khonje et al. (2018), who took non-adopters as the baseline. Table 3 shows the parameter estimation of the MMNL model, which is equivalent to the first stage of the multinomial endogenous treatment effects model. The fitting degree of this model, based on the Wald test, was very good ($\chi^2 = 114.97$, $P > \chi^2 = 0.0041$), indicating that the null hypothesis was rejected.

As mentioned above, the adoption of the additional three technologies was used as the baseline in this study, and the subsequent comparative analysis was based on it.

The results show that the adoption of IPM & BF was positively affected by household size, tobacco farming area, and distance to the township and negatively affected by the proportion of flat land. That is, the larger the household size and tobacco farming area, the more farmers are inclined to adopt the inputs of pesticides and fertilizers. The regression results were significant in terms of the distance to the township, indicating that the farther the distance from the township, the more tobacco farmers tended to adopt IPM & BF, showing that influenced by Chinese planting experience and culture, the farther away from the township, the more tobacco farmers tend to increase investments in pesticides and fertilizers to improve their inner sense of security

The adoption of BF & SI was significantly positively affected by the tobacco farming area and the number of training sessions. It was significantly negatively impacted by education level, years of tobacco farming, distance to a tobacco technology extension station, proportion of flat land, and proportion of leasehold land. The results indicate that tobacco farmers with more area for tobacco farming and more training tended to choose the BF & SI combination. Furthermore, the results indicate that the higher the education level, the higher the tendency of tobacco farmers to adopt IPM & BF & SI than BF & SI. In terms of years of tobacco farming, with increasing years of farming, tobacco farmers were more willing to adopt IPM & BF & SI rather than BF & SI. This finding may be due to a greater belief in the combined application of multiple technologies as years of farming increases. In terms of distance to a tobacco technology extension station, the greater the distance, the more tobacco farmers preferred to adopt IPM & BF & SI, which may be due to a greater distance leading to greater psychological dependence of tobacco farmers on the diversity of technology combinations. In terms of the proportion of leasehold land, tobacco farmers tended to adopt IPM & BF & SI as the proportion of leasehold land increased.

The greater the proportion of flat land, the more tobacco farmers preferred to adopt IPM & BF & SI rather than BF & SI or IPM & BF. It is possible that the greater the proportion of flat land, the easier it is for tobacco farmers to

implement multiple agricultural technologies, which can reduce labor intensity and improve operational efficiency.

In the empirical study, the regression results for age of head of household were non-significant, a finding similar to that in a study of the adoption of CA technologies by Tambo and Mockshell (2018). Our analysis suggested that the possible reason was the low education level of tobacco farmers and a lack of independent knowledge concerning various types of agricultural production technologies, leading to a herd mentality in the adoption of additional agricultural technologies.

Based on the literature related to technology adoption in developing countries, such as those in Africa, credit is an important support mechanism related to technology adoption (Cafer & Rikoon, 2018), but the empirical results in this study showed the variability of loans for farmers is not significant; on the one hand, tobacco farmers may have sufficient funds and thus can generally afford extra technologies, and on the other hand, even if the farmers do not have enough funds, they can easily obtain a special bank loan by virtue of their planting contracts with CTC. These results indicate that funding is not a constraint to technology adoption.

The regression results for the number of household members engaged in tobacco farming were also not significant, which may be due to two reasons. First, CTC promotes adjustments to production organization modes, which deepens the agricultural division of labor, and provides specialized services (access to service economies of scale) in multiple production processes (such as raising seedlings, mechanized farming, plant protection, flue-curing, and grading). Second, it is common for tobacco farming families to hire workers during the busy farming season, making it possible to achieve larger scale planting with a smaller amount of tobacco farming labor.

Notably, in the regression model, the variables of the gender of the head of household and social capital were removed. Based on actual practices, the tobacco technical services provided by CTC to tobacco farmers are universal, and there are no differences in gender or social status.

4.3 Average Treatment Effect of MATs

Table 4 provides the estimated results for the effects of the adoption of additional MATs on YPM, ASPT and IPHL. The results of two normal regressions (second stage) are provided in Table A4 (the regression results for the mixed multinomial treatment effects are not shown to conserve space but are available upon request). For comparison, the regression results were estimated under the assumptions of exogenous and endogenous adoption decision of MATs with the adoption of the three additional technologies as the baseline.

Table 4. Estimation of the multinomial endogenous treatment effects model for the effect of MATs on YPM, ASPT and IPHL in Chongqing

Assumptions	Package	Ln YPM	Ln ASPT	Ln IPHL
Exogenous	IPM only	-0.0566 (0.111)	0.0329 (0.0282)	0.112 (0.148)
	BF only	0.0848*** (0.0317)	-0.0274* (0.0141)	0.0857* (0.0513)
	IPM & BF	0.127 (0.162)	0.0228 (0.0314)	0.171* (0.0990)
	SI only	0.246 (0.154)	0.00532 (0.0573)	0.212 (0.151)
	IPM & SI	-0.00416 (0.0335)	0.0243*** (0.00933)	-0.0159 (0.0447)
	BF & SI	0.104** (0.0414)	0.0381** (0.0173)	0.142* (0.0730)
Endogenous	IPM only	-0.125 (0.129)	0.0465*** (0.00828)	0.268*** (0.0399)
	BF only	0.0770 (0.0501)	-0.0373*** (0.00214)	0.194*** (0.0146)
	IPM & BF	0.205** (0.101)	0.0882*** (0.00343)	0.269*** (0.0263)
	SI only	0.205 (0.125)	0.0157*** (0.00395)	0.170*** (0.0534)
	IPM & SI	-0.0527 (0.0428)	0.00497*** (0.00188)	-0.0708*** (0.0159)
	BF & SI	0.118 (0.108)	0.0750*** (0.00777)	0.248*** (0.0259)
Selection terms(λ)	IPM only	0.0852** (0.0373)	0.0355*** (0.000746)	-0.162*** (0.00499)
	BF only	0.0132 (0.0301)	-0.00978*** (0.000813)	-0.0000220 (0.00456)
	IPM & BF	-0.0940** (0.0441)	-0.0323*** (0.000650)	-0.0199*** (0.00565)
	SI only	0.0563 (0.0344)	0.0428*** (0.000673)	0.231*** (0.00334)
	IPM & SI	0.0723** (0.0333)	0.0389*** (0.000723)	0.130*** (0.00453)
	BF & SI	-0.0125 (0.0332)	-0.0271*** (0.000718)	-0.0707*** (0.00509)

Note. *** $p < 0.01$, ** $p < 0.05$, * $p < 0.1$. Standard errors in parentheses.

The results showed that for adopting the exogenous model of MATs, on average, BF & SI had significant effects on YPM, ASPT and IPHL, while IPM & BF only had a significant impact on IPHL. Compared with adopting all three technologies, the application of BF & SI increased YPM and increased ASPT. Both of them led to an increase in the IPHL (Table 4). However, IPM & BF was only conducive to improving IPHL. The above results are statistical inferences based on the observed characteristics without considering the unobserved influencing factors. Thus, there were endogeneity problems, and the results may be biased. To solve this problem and take into account some of the influencing factors that are not observed, we used a multinomial endogenous treatment effects model.

After the treatment for endogeneity, the average adoption effect when controlling for unobserved heterogeneity showed a difference (Table 4), and the validity of estimation was significantly improved. BF & SI had a significant positive impact on ASPT and IPHL. IPM and BF had a significant positive impact on YPM, ASPT, and IPHL. In terms of value, compared with the adoption of all three technologies, the adoption of BF & SI resulted in an average increase of 7.50% in ASPT and an average increase of 24.80% in IPHL. The adoption of IPM & BF resulted in an average increase of 20.50% in YPM, an average increase of 8.82% in ASPT, and an average increase of 26.90% in IPHL. The combined effects of yield and quality-enhancement led to an increase in IPHL. Notably, although the adoption of IPM & BF significantly increased yield, CTC only purchased the contracted output, and the excess was innocuously disposed of by tobacco farmers. Therefore, the income effect reflected the actual increase in tobacco farmers' income. Compared with the result under the exogenous hypothesis, the estimated value after considering unobservable characteristics was relatively higher, indicating that the actual effect of adoption will be underestimated without consideration of endogeneity.

As seen in Table 4, with the adoption of all three additional technologies as the baseline, the adoption factor load (λ) of BF & SI in the sales price equation and the income equation exhibits negative selection bias, and the factors load (λ) of the yield equation, the sales price equation and the income equation for IPM & BF all show negative selection biases. These results indicated that although the unobservable factors improved the possibility of adopting BF & SI, they had a lower impact on ASPT and IPHL. Unobservable factors can improve the possibility of adopting IPM & BF, while they had a lower impact on YPM, ASPT and IPHL.

Finally, OLS approach is also implemented as a means of robustness checks, results are shown in Table A5. On the whole, the robustness check reveal that the treatments effects discussed above are robust.

5. Conclusions and Implications

5.1 Conclusions

The present study found heterogeneity in factors affecting the adoption of additional different combinations of MATs by tobacco farmers. Specifically, the higher the education level of tobacco farmers, the more years of tobacco farming, the farther the families are from tobacco technology extension stations, and the higher the proportion of flat land and the proportion of leasehold land, the more farmers tend to adopt three additional technology combinations, *i.e.*, the use of pesticides, fertilizers, and soil improvement. The larger tobacco farming area, the larger household size or the farther the distance to a township, the more farmers tend to adopt technology combinations involving pesticides and fertilizers.

The empirical results show that when only observable factors are considered, the estimation of the outcome equation will result in sample selection bias. The empirical results also show some inefficiencies in the adoption behaviour of additional MATs by tobacco farmers. Taking the adoption of three additional technologies as the baseline, the adoption of technology combinations involving pesticides and fertilizers and involving fertilizers and soil improvement had a significant positive impact on the IPHL. Furthermore, the adoption of technology combinations involving pesticides and fertilizers also had a significant positive impact on both YPM and ASPT, indicating that a yield effect and quality-enhancement effect were produced, and the positive impact on IPHL was relatively greater. We found that although most tobacco farmers adopt a combination of the three additional technologies, it was not the best income choice. Some researchers has reported similar findings. For example, Di Falco and Veronesi (2013) studied the strategies adopted by farmers in the Nile basin in Ethiopia to adapt to climate changes, Manda et al. (2016) studied the adoption of SAPs by farmers in Zambia, and Ng'ombe, Kalinda, and Tembo (2017) studied the adoption of conservation farming (CF) practices by farmers in Zambia. This issue deserves constant attention.

5.2 Policy Implications

Based on our research conclusions, we can infer some meaningful policy implications. First, the adoption of additional pesticide and fertilizer combination can not only significantly improve the yield per mu of tobacco, but also significantly improve the quality grade of tobacco, which can increase the sales revenue of tobacco farmers. Therefore, tobacco farmers should put in additional pesticides and fertilizers appropriately based on the implementation of standardized technology system while considering the specific characteristics of the plot and pest situation. Second, because technical training can significantly increase the professional knowledge of tobacco farmers and reduce inefficient additional technology investments, CTC should further strengthen training for tobacco farmers, especially for tobacco farmers far away from tobacco technology extension stations. Third, the combination of fertilizer and soil improvement technology is the second highest income scheme just after the combination of pesticide and fertilizer, although the investment of soil improvement technology is comparatively large and the return period is long, the negative externality of environment is small. The government should give tobacco farmers technical subsidies for soil improvement, stabilize land lease term and other policy support, in order to promote tobacco farmers to adopt soil improvement technology and give overall consideration between the current income growth and sustainable development.

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Appendix A

Table A1. Distribution of questionnaires in 12 districts and counties

District/County	Number of questionnaires(n)	Valid questionnaires(n)	Percentage proportion (%)
Youyang County	65	56	86.15%
Wulong District	55	45	81.82%
Pengshui County	90	55	61.11%
Fuling District	15	13	86.67%
Qianjiang District	45	33	73.33%
Nanchuan District	10	10	100%
Fengdu County	35	24	68.57%
Shizhu County	30	17	56.67%
Wushan County	60	38	63.33%
Wuxi County	35	35	100%
Fengjie County	45	43	95.56%
Wanzhou District	15	15	100%
Total	500	384	76.80%

Note. n: sample size.

Table A2. Descriptive statistics by IPM & BF, BF & SI, IPM & BF & SI

Variables	IPM & BF	BF & SI	IPM & BF & SI
	---------- Mean ----------		
YPM	117.669 (2.594)	112.614 (2.848)	112.196 (1.734)
ASPT	26.937 (0.338)	28.347 (0.205)	27.701 (0.137)
IPHL	6.677 (0.657)	5.82 (0.444)	5.966 (0.26)
Gender	0.9 (0.048)	0.929 (0.031)	0.932 (0.016)
Age	50.55 (1.129)	49.343 (0.785)	49.841 (.449)
Education	2.75 (0.123)	2.5 (0.073)	2.701 (0.047)
Years	22.15 (1.721)	16.443 (0.997)	21.263 (0.63)
Homesize	5.325 (0.249)	4.771 (0.154)	4.869 (0.091)
Planter	2.2 (0.114)	2.143 (0.089)	2.048 (0.036)
Area	43.825 (3.97)	38.443 m(3.128)	37.496 (1.365)
Distance1	11.803 (1.606)	6.086 (0.504)	8.89 (0.437)
Loan	0.425 (0.079)	0.414 (0.059)	0.442 (0.031)
Train	5.325 (0.439)	6.086 (0.285)	4.952 (0.152)
Labour	0.683 (0.035)	0.689 (0.028)	0.7 (0.015)
Distance2	6.1 (0.724)	3.167 (0.335)	5.759 (0.327)
Ground	0.657 (0.046)	0.657 (0.04)	0.749 (0.018)
Rentedfarmland	0.674 (0.051)	0.571 (0.039)	0.697 (0.036)
Observations	40	70	251

Note. Standard errors in parentheses.

Table A3. Test on the validity of selection instruments

Variables	IPM & BF	BF & SI	IPM & BF & SI
Ln YPM	F(3, 26) = 0.25	F(3, 56) = 1.53	F(3, 237) = 0.32
Ln ASPT	F(3, 26) = 0.66	F(3, 56) = 0.68	F(3, 237) = 0.40
Ln IPHL	F(3, 26) = 0.96	F(3, 56) = 1.97	F(3, 237) = 1.98
Observations	40	70	251

Table A4. Second stage estimates for YPM, ASPT and IPHL

Variables	Ln YPM	Ln ASPT	Ln IPHL
Age	-0.00192 (0.00196)	-0.00162*** (0.0000994)	-0.00511*** (0.000650)
Education	-0.00631 (0.0185)	0.00419*** (0.000909)	0.0408*** (0.00564)
Years	-0.000567 (0.00139)	0.000747*** (0.0000743)	-0.00506*** (0.000505)
Homesize	-0.0000780 (0.0106)	0.00263*** (0.000523)	0.0133*** (0.00328)
Planter	-0.0202 (0.0212)	-0.0112*** (0.00117)	-0.515*** (0.00573)
Area	-0.00166*** (0.000604)	0.0000775** (0.0000309)	0.0197*** (0.000174)
Distance1	-0.00257 (0.00182)	-0.000321*** (0.0000936)	-0.00305*** (0.000656)
Loan	-0.0213 (0.0271)	-0.0242*** (0.00147)	0.0170* (0.00978)
Train	0.00522 (0.00515)	0.00616*** (0.000234)	0.0146*** (0.00219)
Labour	-0.0694 (0.0628)	0.0397*** (0.00339)	-0.0374 (0.0231)
Constant	4.982*** (0.137)	3.334*** (0.00708)	2.011*** (0.0420)

Note. *** $p < 0.01$, ** $p < 0.05$, * $p < 0.1$. Standard errors in parentheses.

Table A5. Robustness test results

Model	OLS		
Package	Ln YPM	Ln ASPT	Ln IPHL
IPM & BF	0.082*** (2.567)	-0.032** (-2.30)	0.088* (1.72)
BF & SI	-0.014 (-0.43)	0.029*** (3.24)	-0.024 (-0.55)
IPM & BF & SI	-0.028 (-1.09)	-0.006 (-0.65)	-0.024 (-0.68)

Note. *** $p < 0.01$, ** $p < 0.05$, * $p < 0.1$. z-values are in parentheses. Due to the implementation of tobacco production contract management，the values of ASTP in the table were affected to some extent, robustness check can be judged just by values of YPM and IPHL.

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Effect of Method of Application, Herbicide Rate and Cultivar on Processing Pea Tolerance to Saflufenacil

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Abstract

The purpose of this work was to determine the effect of method of application, herbicide rate and cultivar on tolerance of processing pea tolerance to saflufenacil. Two field experiments were established to address this—each experiment was conducted over a 3-year period. The first experiment, conducted in 2014, 2015 and 2016, was arranged in a split-plot design with method of application (pre-plant incorporation (PPI) or preemergence (PRE)) as the main plot factor, and saflufenacil rate (0, 75 and 150 g ai ha^{-1}) as the subplot factor. Pea (*Pisum sativum* L.) was not injured, and dry matter, pea tenderness and yield were not less than the untreated check when saflufenacil was applied either PPI or PRE, at 75 and 150 g ai ha^{-1} of the herbicide. The second experiment was conducted from 2017 to 2019, at two locations each year; each repetition of this experiment was arranged in a factorial design to determine the effect of two factors on processing pea: saflufenacil rate (0, 75 and 150 g ai ha^{-1}) and cultivar. Saflufenacil did not cause more than 5% visible injury to pea, nor did it reduce pea dry matter, tenderness or marketable yield of the eight cultivars included in the experiment. Application method, saflufenacil rate and cultivar did not affect pea tolerance across a wide range of soil and environmental conditions. Registration of saflufenacil in processing pea would significantly improve growers' options for control of Group 2 resistant broadleaf weeds such as common lamb's-quarters (*Chenopodium album* L.), eastern black nightshade (*Solanum ptycanthum* Dunal.) and common ragweed (*Ambrosia artemisiifolia* L.).

Keywords: cultivar sensitivity, herbicide tolerance, pre-plant incorporation, preemergence, *Pisum sativum* L., saflufenacil

1. Introduction

In the province of Ontario, succulent field pea (*Pisum sativum* L.) grown for the canning and frozen pea markets offers field crop growers an opportunity to diversify farm operations and increase overall profit on farm. Approximately 6,200 ha of succulent pea were grown in Ontario in 2016, this high value crop provided on-farm income of $11.8 M (OMAFRA, 2017). Pea is considered to be a poor competitor, since cultivars grown for these markets are semi-leafless (*i.e.*, tendrils replace leaflets at shoot tips), so they intercept less light than leafy cultivars (Martin et al., 1994; Semere & Froud-Williams, 2001). Effective control of weeds is an important part of successful production of succulent pea.

The most problematic weeds pea growers must control are annual broadleaf weed species. This includes species such as eastern black nightshade (*Solanum ptycanthum* Dunal.), common lamb's-quarters (*Chenopodium album* L.) and common ragweed (*Ambrosia artemisiifolia* L.). These weeds can reduce yield of pea as much as 67%, interfere with harvest, and in extreme circumstances result in results in not harvesting some fields (Lutman et al., 1994; Townley-Smith & Wright, 1994). Management of these weeds in succulent pea is further complicated by the development of resistance a commonly used broadleaf herbicide, imazethapyr (OMAFRA, 2016), an herbicide the inhibits the function of the acetolactate synthase (ALS) enzyme (Shaner et al., 1984). The prevalence of annual broadleaf weed species resistant to ALS-inhibiting herbicides (*i.e.*, Group 2 herbicides) provides a basis for including herbicides with alternate modes-of-action that are capable of controlling these problematic weeds as part of a management strategy.

Saflufenacil is a preemergence herbicide registered in corn and soybean that controls a wide range of annual weeds (Grossmann et al., 2010), including Group 2 resistant biotype of those species mentioned in the previous paragraph. Saflufenacil is a pyrimidinedione, a Group 14 herbicide that inhibits protoporphyrinogen-IX oxidase

(PPO) in the chloroplast, ultimately leading to the accumulation of singlet oxygen radicals in the cytoplasm, which disrupt the cell membrane (Grossman et al., 2010). Since saflufenacil has a different mode-of-action than imazethapyr, it could potentially offer growers a means to control Group 2-resistant biotypes of eastern black nightshade, common lamb's-quarters and common ragweed in succulent pea. While preliminary research suggests that processing pea has tolerance to soil applications of saflufenacil (Soltani et al., 2010), the effect of application method and pea cultivar has yet to be reported upon in the literature.

Incorporation may affect pea response to soil applied herbicides. Incorporation generally will improve control in growing season when rain fails to fall within a couple of weeks of application, because it moves the herbicide into soil water solution (Sikkema & Robinson, 2005). However, incorporation also moves the herbicide into the zone where crop root development begins. In pea, incorporation resulted in injury and yield loss when planted after *s*-metolachlor; however, when the herbicide was left undisturbed on the soil surface, injury and yield loss were not observed (Sikkema & Lambgrets, 1995). The first objective of this research was to develop an understanding of the effect of incorporation on pea response to saflufenacil.

The substantial genetic diversity that exists among pea cultivars grown in North America (Tar'an et al., 2005; Kwon et al., 2011) is an important consideration in any attempt to characterize pea response to herbicides. Some research has shown that little variation in herbicide sensitivity exists among pea cultivars to bentazon and MCPA (Jensen, 1993); however, Hicks (2003) showed pea cultivars possessed varying levels of sensitivity to PRE applications of cyanazine, metribuzin, terbuthylazine and trifluralin. These latter findings suggest that understanding herbicide tolerance in pea should include a range of cultivars.

Though differences in cultivar sensitivity to saflufenacil in pea are unknown, the response of other important crops to this herbicide has been elucidated. Miller et al. (2012) demonstrated differences in soybean (*Glycine max* L.) emergence, visible injury, plant dry weight and yield of 12 soybean cultivars planted after PRE applications of 25 to 200 g ai ha^{-1} saflufenacil. In contrast, sweet maize (*Zea mays* L.) hybrids possessed no differential response to PRE applications of saflufenacil at rates ranging from 75 to 150 g ai ha^{-1} (Robinson et al., 2012). The second objective of this research was to determine whether response to soil applied saflufenacil varied among eight commercially-grown pea cultivars.

2. Materials and Methods

2.1 Experiment 1: Influence of Application Method and Herbicide Rate on Processing Pea Tolerance to Saflufenacil

The first experiment was conducted on a Maplewood/Normandale sandy clay loam with 54% sand, 25% silt, 21% clay, 5.2% organic matter and pH of 6.7 in 2014, a Normandale sandy loam with 58% sand, 24% silt, 18% clay, 3.2% organic matter and pH 6.9 in 2015, and a Watford Brady sandy clay loam with 52% sand, 28% silt, 20% clay, 5.9% organic matter and pH of 7.2 in 2016. Each site was mouldboard plowed in the fall of year prior to establishment, and fertilizer was applied to provide 38 kg ha^{-1} of actual nitrogen. Phosphorous and potassium were amended as needed based on soil tests. The sites were then worked once with an S-tine cultivator prior to planting.

The experiment was arranged in a split-plot design with all treatments replicated four times. Main plots were 8.0 m wide by 8.0 m long, and each sub-plot was 2.0 m wide by 8.0 m long—a 1.0 m wide untreated boundary was left between each sub-plot. The main plot factor was method of application (pre-plant incorporation (PPI) or preemergence (PRE)), and the subplot factor was saflufenacil rate (0, 75 and 150 g ai ha^{-1}). The rates were chosen based upon the current dose registered in maize: 75 g ai ha^{-1}. Herbicides were applied two to three days before pea planting with a CO_2-pressurized backpack sprayer calibrated to deliver 210 L ha^{-1} spray solution at 200 kPa using Teejet 8002 flat-fan nozzles (Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60188). The boom was 2.0 m long with five nozzles spaced 50 cm apart. Incorporation of the PPI treatments was done the day of application with two passes in opposite directions of an S-tine cultivator with rolling basket harrows. 'Spring' pea was planted to a depth of 5.0 cm using 18- and 5-cm between- and within-row spacings, respectively, at a rate of 300 kg seed ha^{-1}. Pea was planted on 2 April, 2014, 8 April, 2015 and 9 April, 2016. All plots were hand-weeded as required until pea vines covered the inter-rows.

Crop injury was rated 1, 2 and 4 weeks after pea emergence (WAE) on a scale of 0 to 100%. A rating of 0 denoted no visible plant injury, while 100 indicated complete plant death. At 4 WAE, ten plants were randomly sampled from each plot and placed into a paper bag, dried for four days at 60 ^{o}C, and dry weight of the ten plants combined was determined. As pea approached maturity, a random sample of deshelled pea fruit from ten pea pods in the untreated strips between plots was collected each day. Tenderometer readings of these samples were made using a tenderometer (TU-12, manufactured by Food Technology Corporation, 45921 Maries Road, Sterling, VA 20166), to determine when pea was ready to harvest. Pea was harvested when tenderometer

readings in the untreated strips reached 100 tenderness units (TUs), a value considered optimal for processing (*i.e.*, mature), and thus ready for harvest (Everaarts & Sukkel, 2000). At maturity, two 1 m^2 quadrats of plants were harvested by hand from each plot and combined, immediately put through a stationary pea thresher to separate pea fruit from the shells, and fresh weight of deshelled pea fruit was measured. From this, a 50 g sample of pea fruit was placed into the tenderometer to determine pea tenderness.

The general linear mixed model (GLIMMIX) of SAS 9.3 (SAS 2011) was used to analyze fixed effects of application method, saflufenacil rate, the interaction between fixed effects, as well as random effects of year, and the year-by-treatment interactions. Shapiro-Wilk, Durban-Watson and Levene's tests were used to confirm normality, independence of errors and homogeneity of variances. Percent injury data were not normal, so estimates and standard errors were obtained using the logit ILINK. Plant dry weight, tenderometer readings and yield at maturity met all three assumptions of analysis of variance. Tukey's HSD test ($\alpha = 0.05$) was used to separate treatment means.

2.2 Experiment 2: Influence of Cultivar and Herbicide Rate on Processing Pea Tolerance to Saflufenacil

A second experiment examined the interaction between pea cultivar and saflufenacil rate from 2017 to 2019. Each year, the experiment was established at two randomly chosen locations at the Ridgetown Campus. In 2017, the soil at one of the locations was a Normandale loamy fine sand (78% sand, 13% silt, 9% clay; 3.5% OM, pH 6.6 and CEC 8 meq 100 g^{-1} soil), and at the second site was a Watford Brady clay loam (38% sand, 30% silt, 32% clay; 4.3% OM, pH 6.9 and 14 meq 100 g^{-1} soil). In 2018, the experiment was established on a Watford Brady loam (50% sand, 28% silt, 22% clay; 4.7% OM, pH 6.0 and 12 meq 100 g^{-1} soil) and Normandale loamy fine sand (82% sand, 10% silt and 8% clay; 3.9% OM, pH 6.5 and 7 meq 100 g^{-1} soil). In the third year, soil types were Watford Brady loam (46% sand, 27% silt and 27% clay; 4.2% OM, pH 7.1 and 12 meq 100 g^{-1} soil) and Normandale very fine sandy loam (72% sand, 15% silt, 13% clay; 5.4% OM, pH 7.1 and 11 meq 100 g^{-1} soil). All sites were plowed, fertilized and seedbed prepared as in the first experiment. Pea was planted to a depth of 5.0 cm using 18- and 5-cm between- and within-row spacings, respectively, at a rate of 300 kg seed ha^{-1}. Pea was planted on 4 April, 2017, 12 April, 2018 and 6 April, 2019. All plots were hand-weeded as required until pea vines covered the inter-rows. Plots were 2 m wide by 8 m long, and the experiment was arranged in a factorial design with two factors. The first factor was saflufenacil rate (0, 75 and 150 g ai ha^{-1}), and the second factor was pea cultivar ('Tyne, 'Sweet Savour', 'Reliance', 'Lil Mo', 'Gallant', 'Salerno', 'Naches', 'Spring'). The cultivars were chosen to reflect a diversity of different genotypes of cultivars grown commercially in the province of Ontario. Herbicides were applied PRE, two to three days before planting, using the same application procedure and equipment from Experiment 1. All plots were kept weed-free with hand-weeding.

Data collection, confirmation of assumptions of ANOVA and statistical procedures were conducted using the same methods as in Experiment 1. As in the first experiment, the logit ILINK was to obtain estimates of percent injury. In this experiment, fixed effects were saflufenacil rate, pea cultivar, and the interaction between the two variables, while random effects of year and year-by-treatment interactions were also included in the analysis.

3. Results and Discussion

3.1 Experiment 1: Influence of Application Method and Herbicide Rate on Pea Tolerance to Saflufenacil

Mean monthly temperature and precipitation data during the course of this study are shown in Table 1. Pea response to soil applications of saflufenacil was combined over years and locations, as there were no interactions between random effects of year and fixed effects of application method or herbicide rate. Across years, rainfall in the month of April ranged from 32 to 102 mm, and mean monthly soil temperature varied from 6.2 to 7.0 ^{o}C (Table 1), suggesting that pea tolerance to PRE herbicides was consistent among years, despite variable soil moisture and temperature levels.

Table 1. Monthly rainfall and mean daily temperature in each year (2014 to 2016) of the method of application experiment (Experiment 1) from the start of the month of herbicide application to the end of the month of crop harvest, as well as 30-yr averages for monthly rainfall and daily mean temperature at Ridgetown, Ontario.

Month	2014		2015		2016		30-year average	
	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)	Rainfall (mm)	Temperature (°C)
April	32	7.0	102	6.2	66	6.6	76	7.8
May	34	15.5	64	15.1	97	13.4	80	14.0
June	45	22.6	102	18.6	48	19.8	70	19.2
July	156	22.1	78	21.2	130	19.1	74	21.6

Note. Monthly precipitation and mean temperature data were obtained from the on-site weather station located at Ridgetown, Ontario.

Visible injury and dry weight of ten plants per plot were similar to the untreated check treatments in all instances. Pea visible injury ranged from 0 to 3% and 0 to 4% in the pre-plant incorporated (PPI) and preemergence (PRE) treatments as saflufenacil rate increased from 0 to 150 g ai ha^{-1}, respectively (Table 2). There was not an effect of application method ($p = 0.2022$), saflufenacil rate ($p = 0.5059$) or their interaction ($p = 0.1397$) on visible injury in pea. Corresponding to the lack of visible injury observed, dry weight was not influenced by application method ($p = 0.2681$), herbicide rate ($p = 0.4720$), or their interaction ($p = 0.901$). Dry weights were 10.7 and 11.3 g $plant^{-1}$ at saflufenacil rates of 0 and 150 g ai ha^{-1} in the PPI treatments, and 11.4 and 11.0 g $plant^{-1}$ at 0 and 150 g ai ha^{-1} rates of saflufenacil (Table 2), respectively, in the PRE applications. These results are similar to those of Soltani et al. (2010), who found that PRE applications of saflufenacil neither injured nor reduced growth of pea at rates of 100 and 200 g ai ha^{-1}.

Table 2. Effect of method of application and saflufenacil rate on pea visible injury at 28 days after emergence (DAE), average dry weight of ten plants per plot, tenderometer reading at harvest and shelled yield averaged over three years (*i.e.*, 2014 to 2016) and two locations at Ridgetown, Ontario

Method of Application	Saflufenacil Rate (g ai ha^{-1})	Visible Injury (%)	Dry Weight (g $plant^{-1}$)	Tenderometer Reading (TUs)	Yield (T ha^{-1})
Pre-plant Incorporation	0	0	10.7 a	100 a	4.5 a
	75	1 a	10.8 a	100 a	4.2 a
	150	3 a	11.3 a	100 a	4.8 a
Premergence	0	0	11.4 a	100 a	4.8 a
	75	2 a	11.7 a	104 a	4.6 a
	150	4 a	11.0 a	101 a	4.5 a
Standard Error		4	1.3	7	0.6

Note. Pea tenderometer readings are measured in tenderness units (TUs) on a scale of 0 to 160, which is used by the processing pea industry to estimate maturity. Tenderometer readings of 100 to 105 are optimal at harvest.

In addition to no effect of application method or saflufenacil rate on pea visible injury and dry matter production, pea tenderometer readings and marketable yield also were unaffected. Pea tenderness was 100 and ranged from 100 to 104 tenderness units at saflufenacil rates of 0 and 150 g ai ha^{-1} in the PPI and PRE application timings, respectively (Table 2). Application method ($p = 0.1598$), saflufenacil rate ($p = 0.0884$) and the interaction between them ($p = 0.1843$) did not have an effect on pea tenderness. This has important implications for making harvesting decisions, as tenderness is considered an accurate estimate of fresh pea maturity (Wisscher & Lovink, 1999). Pea yield varied from 4.2 to 4.8 t ha^{-1} and from 4.5 to 4.8 t ha^{-1} in the PPI and PRE application timings, respectively. Pea yield was not influenced by application method ($p = 0.2555$), saflufenacil rate ($p = 0.3807$), or the interaction between application method and saflufenacil rate ($p = 0.8941$). Similar to what was observed in for pea injury and dry matter, pea tenderness and yield were not different from the untreated controls.

This experiment showed pea responded similarly to the untreated check, when saflufenacil was applied either PPI or PRE, at 75 and 150 g ai ha^{-1} of the herbicide. This is in contrast to previous work with *s*-metolachlor, which caused significant injury to pea when incorporated, but not when left on the soil surface (Sikkema &

Lambgrets, 1995), and that injury increased with herbicide rate in PPI applications. The differential response of pea to these herbicides may occur because differential metabolism, though differential uptake may be partially responsible for this finding. Saflufenacil is taken up through the root (Frihauf et al., 2010), while *s*-metolachlor is absorbed through the elongating hypocotyl as well as roots (Deal & Hess, 1980). Incorporation places the herbicide into the region of the soil where hypocotyls grow, which may therefore increase the potential for injury from *s*-metolachlor but not saflufenacil.

The results and limitations of this experiment were used to guide the design of the second experiment on different pea cultivars. Since application method (PPI versus PRE) did not affect pea tolerance to saflufenacil, and given that pea growers in Ontario generally prefer not to incorporate soil applied herbicides due to the extra time, fuel and equipment needed, the second experiment was conducted using only PRE applications of saflufenacil. One limitation of Experiment 1 was the inclusion of a single pea cultivar, which limits the applicability of the results to a relatively small portion of the genome among commercially-grown pea cultivars (Tar'an et al., 2005). We did not find prior research that examined whether pea cultivar influences tolerance to saflufenacil; however, tolerance to trifluralin differed among pea cultivars (Hicks, 2003). Furthermore, Miller et al. (2012) observed variable tolerance to saflufenacil among soybean cultivars. As a result, a second experiment was planned to include several pea cultivars.

In addition to the limitation of a single cultivar, Experiment 1 was only conducted on one soil type in each year of study, which also limits the applicability of the results. Gannon et al. (2014) showed that saflufenacil toxicity in canola (*Brassica napus* L.) was strongly correlated ($r = 0.85$) to soil organic matter. This is common for other soil-applied, residual herbicides with the same mode-of-action, such as sulfentrazone (Grey et al., 1997) and flumioxazin (Ferrell et al., 2005). As a result, further analysis of the response of pea to saflufenacil was conducted on different soil types and weather conditions.

3.2 Experiment 2: Influence of Saflufenacil Rate and Cultivar on Pea Tolerance to Saflufenacil

Mean monthly temperature and monthly precipitation from 2017 to 2019 are presented in Table 3. Since random effects of site, year, and their interactions with either application method or saflufenacil rate were not significant, data were combined over sites and years. The fact that data could be combined across all sites and years is notable, given that the amount of rainfall in April varied from 54 to 113 mm, and mean soil temperature ranged from 5.8 to 10.1 ^{o}C; (Table 3). In addition, the experiment was repeated on a variety of soil textures, ranging from a loamy fine sand to clay loam soils, organic matter (3.5 to 5.4%), pH (6.0 to 7.1) and CEC (7 to 14 meq 100 g^{-1} soil), suggesting that pea cultivar response to PRE herbicide applications of saflufenacil was consistent across the range of environments experienced in the trials.

Table 3. Monthly rainfall and mean daily temperature in each year (2017 to 2019) of the pea cultivar experiment (Experiment 2) from the start of the month of herbicide application to the end of the month of crop harvest, as well as 30-yr averages for monthly rainfall and daily mean temperature at Ridgetown, Ontario

Month	2017		2018		2019		30-year average	
	Rainfall (mm)	Temperature (^{o}C)	Rainfall (mm)	Temperature (^{o}C)	Rainfall (mm)	Temperature (^{o}C)	Rainfall (mm)	Temperature (^{o}C)
April	54	7.2	92	5.8	113	10.1	76	7.5
May	90	16.2	106	13.8	102	12.6	80	13.9
June	98	18.1	51	18.9	45	19.7	70	19.2
July	62	19.8	79	21.8	36	21.2	74	21.4

Note. Monthly precipitation and mean temperature data were obtained from the on-site weather station located at Ridgetown, Ontario.

Visible injury and dry weight of pea in the herbicide treated plots were similar to the untreated check treatments, for all cultivars across all sites and years. Pea visible injury was less than 10% in all treatments (Table 4). There was not an effect of pea cultivar ($p = 0.2511$), saflufenacil rate ($p = 0.2527$) or their interaction ($p = 0.6481$) on visible injury in pea. Pea dry weight was not reduced by PRE applications of saflufenacil at either 75 or 150 g ai ha^{-1} ($p = 0.9400$). Dry weights varied from 10.8 to 14.5 g $plant^{-1}$ (Table 4) across all cultivars ($p = 0.0081$), but the interaction between saflufenacil rate and cultivar was not significant ($p = 0.2635$). The difference in plant dry

weight may among pea cultivars in this trial is not unexpected, and is likely explained by the significant variability in the genome among pea cultivars grown in Canada (Ta'ran et al., 2005).

Table 4. Effect of pea cultivar on visible injury at 28 days after emergence (DAE), average dry weight of ten plants per plot, tenderometer reading at harvest and shelled yield averaged over three years (*i.e.*, 2017 to 2019) and two locations at the Ridgetown Campus field research station in Ridgetown, Ontario

Cultivar	Visible Injury (%)	Dry Weight (g plant^{-1})	Tenderometer Reading (TUs)	Yield (T ha^{-1})
Salerno	4 a	12.8 b	100 a	3.3 b
Gallant	4 a	14.5 a	101 a	3.8 ab
Lil Mo	3 a	10.8 d	100 a	3.8 ab
Naches	4 a	12.8 b	101 a	2.8 c
Reliance	2 a	12.6 b	100 a	3.6 b
Spring	2 a	12.2 bc	101 a	4.1 a
Sweet Savour	4 a	11.6 cd	100 a	3.2 bc
Tyne	0 a	13.8 a	102 a	3.3 b
Standard error	5	0.8	7	0.3

Note. Within each column, means that are different than one another are denoted by different letters according to Tukey's test ($\alpha = 0.05$).

In addition to having no negative effect on pea visible injury or dry matter production, saflufenacil rate did not reduce pea tenderometer readings or marketable yield. Pea tenderness was similar across all three saflufenacil rates ($p = 0.8908$), and the interaction between pea cultivar and saflufenacil rate was not significant ($p = 0.9929$); indicating that herbicide treatments did not alter maturity among any of the pea cultivars in the experiment. Pea yield varied among cultivars ($p < 0.0001$); yield ranged from 2.8 in 'Naches' to 4.1 t ha^{-1} in 'Spring' (Table 4). This difference among pea cultivars is likely due to genomic differences that exist among pea cultivars grown in Canada (Ta'ran et al., 2005). Pea yield was not different between the untreated check and either rate of saflufenacil ($p = 0.2070$), and was unaffected by the interaction between pea cultivar and saflufenacil rate ($p = 0.9332$).

4. Conclusion

The purpose of this work was to determine how method of application, saflufenacil rate, and pea cultivar affected the tolerance of processing pea to saflufenacil. Saflufencil was selected for this work because of its efficacy on Group 2 resistant biotypes of common lambsquarters, eastern black nightshade and redroot pigweed (Grossmann et al., 2010), all of which are prevalent in the processing pea production region of Ontario. Additionally, previous work (Soltani et al., 2010) suggested a good level of tolerance in processing pea might exist. The main limitation of prior research was that it was conducted on a single cultivar, and on a limited range of soil types. The research presented in this paper shows that tolerance to saflufenacil in processing pea was not affected by application method (ie. pre-plant incorporated versus preemergence), cultivar, or saflufenacil rate up to 150 g ai ha^{-1} under a wide range of weather and soil conditions. Currently, saflufenacil is registered in field pea in Canada, and a minor use submission is being prepared to register this herbicide in processing pea. The work has significant implications for the management of Group 2-resistant broadleaf weeds, which are difficult-to-control in processing pea, limit yield and may impede harvest (Lutman et al., 1996). The knowledge that application method and cultivar did not affect pea tolerance under a wide range of environmental conditions, increases our confidence that this herbicide would be a safe, effective option to manage several broadleaf weeds in this important crop.

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Comparison of Soil Biological Properties and Bacterial Diversity in Sugarcane, Soybean, Mung Bean and Peanut Intercropping Systems

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Abstract

Sugarcane intercropping with soybean [*Glycine max* (Linn.) Merr.], mung bean [*Vigna radiata* (Linn.) Wilczek] and peanut (*Arachis hypogaea* Linn.) as well as a sugarcane monoculture were conducted to study the impacts of intercropping on soil biological characteristics and bacterial diversity. The results showed that soil cultivable microorganisms, the activities of soil enzymes and microbial biomass carbon, nitrogen, and phosphorus were all significantly improved by intercropping with soybean and mung bean. Additionally, soil bacterial diversity and richness in sugarcane fields were also significantly enhanced by intercropping with soybean and mung bean. In addition, soil bacterial community structures in sugarcane fields can be altered by intercropping with different legumes. Proteobacteria, a high-nutrient-tolerant bacterial assemblage, became the dominant bacteria in the sugarcane-soybean and sugarcane-mung bean intercropped soils. Twenty four, 28, 26 and 27 dominant soil bacterial genera were found after the sugarcane-soybean, sugarcane-mung bean, sugarcane-peanut and sugarcane monoculture treatments, respectively. Sugarcane-mung bean intercropping being the most promising system for regaining and improving soil fertility and soil heath and facilitate agriculture intensification of sugarcane.

Keywords: sugarcane (*Saccharum officinarum* L.), legume crop, soil enzymes, bacterial diversity

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is the primary source of sugar and is also utilized as a major biofuel and bioenergy crop worldwide (Tomes et al., 2011; Chandel et al., 2012). China is the third largest sugar producing country in the world after Brazil and India. In China, approximately 90% of sugarcane crops are planted in the southern and southwest regions, which are mainly in Guangxi, Guangdong, and Yunnan Provinces. Among these provinces, Guangxi Province is the top sugarcane and sugar producer and accounts for more than 65% of the total sugar production in China (Li, 2004). In China, sugarcane production is largely confined to hilly terrain under rainfed conditions that result in relatively low yields. The problem is worsened by the long-term overuse of chemical fertilizers and pesticides to improve cane and sugar yields (Robinson et al., 2011). For example, higher amounts of N fertilizer, as high as 600-800 kg N ha^{-1} in some regions, are applied annually to sugarcane crops in China, while only 60-120 kg N ha^{-1} are applied in Brazil (Li & Yang, 2014). Long-term chemical fertilizer overuse negatively influences soil microbial ecology and terrestrial and aquatic ecosystem function

(Robertson & Vitousek, 2009). Therefore, minimal chemical fertilizer inputs for maintaining healthy soil and high crop productivity are urgently needed for commercial sugarcane production in China.

Intercropping, which involves growing two or more crop species simultaneously in the same field, is an ancient cropping system that is practiced all around the world (Solanki et al., 2016). Intercropping contributes to the ecofunctional and sustainable intensification of crop production (Raseduzzaman & Jensen, 2017) and is considered an efficient way to achieve agriculture sustainability (Vandermeer, 2011). At present, intercropping is more common in developing countries than developed countries and is practiced mostly by small and subsistence farmers (Sileshi et al., 2012). Intercropping enables agricultural intensification, which delivers higher yields per unit area and increases resource use efficiency compared with monoculture crops (Hauggaard-Nielsen et al., 2008). In particular, the application rates of synthetic nitrogen fertilizer can be reduced by legume intercropping owing to its capacity for biological nitrogen fixation. Moreover, intercropping promotes biodiversity in cropping systems and causes them to be more resilient when faced with environmental stresses, diseases and pests (Frison et al., 2011; Brooker et al., 2014). However, not all intercropping systems deliver yield benefits or other positive outcomes. For example, some cereal-legume intercropping methods produce lower biomass and nitrate accumulations in soil than that of monoculture crops (Li et al., 2001; Luo et al., 2016). Recently, some studies have compared intercropping to monocultures by focusing mainly on weed control, management factors, intercrop productivity, and resource use efficiency (Weerarathne et al., 2017; Yu et al., 2015, 2016; Pelzer et al., 2014). However, little is known about the effects of different intercrops on soil quality, particularly soil biology and related processes in China.

Soil quality depends on a large number of physical, chemical, biological, biochemical and microbiological parameters (Chaer et al., 2009). In particular, the latter two are the most sensitive indicators and respond rapidly to changes (Bastida et al., 2008). Soil enzyme activity is capable of reflecting ecosystem processes (Doran & Zeiss, 2000). In addition to enzymatic activity, soil microbial biomass carbon (MBC), microbial biomass nitrogen (MBN) and microbial biomass phosphorus (MBP) are also used to monitor soil quality (Pandey et al., 2014). Soil microorganisms play an important role in soil biogeochemical processes, such as nitrogen, phosphorus and other element cycles (Urbanová et al., 2015). It is now recognized that soil microbial community composition and diversity determine soil health and crop productivity to a great extent (Mangan et al., 2010).

Therefore, in the present study, we investigated soil fertility and soil bacterial diversity under different sugarcane-legume intercropping systems, which are an important but overlooked aspect of the very promising crop diversification systems in China.

2. Method

2.1 Field Site Description and Experimental Designs

Field experiments were carried out in the 2016-17 and 2017-18 crop seasons at the experimental farm of the Guangxi South Sub-tropical Agricultural Science Research Institute, Longzhou (106°47′34″E and 22°19′42″N). The experimental site locates in southern subtropical monsoon climate zone, which is rich in sunshine and abundant rainfall. And it is slightly cold in winter and spring, hot and rainy in summer, warm and cool in autumn, distinct dry and wet seasons. The average annual temperature is around 22 °C, and the annual precipitation is around 1273.6 mm. Experiments were conducted using a randomized block design with three replications to study the performances of a sugarcane monoculture as control and sugarcane intercropping treatments with soybean [*Glycine max* (Linn.) Merr.], mung bean [(*Vigna radiata* (Linn.) Wilczek], or peanut (*Arachis hypogaea* Linn.) with a 2:2 design (two rows of soybean, mung bean or peanut planted between each sugarcane row). Total of 4 treatments, each treatment set up 3 replications, a total of 12 plots, each plot 5 rows, row length 7 m, sugarcane cultivation row spacing 1.8 m, plot area 63 m^2. The experimental land was plowed and harrowed using a tractor to open rows and then planted. Soya beans (variety name: Gui Chun 10), mung beans (variety name: Medium Green 8) and peanuts (variety name: Gui Hua 1026) were planted between the sugarcane (variety name: ROC 22) rows. All intercropping treatments were all managed in the same conventional method.

2.2 Soil Sampling and Soil Biological Properties Analysis

Soil samples were collected in July 2018 from 12 plots that represented all the treatments in the intercropping experiments. To collect soil samples, the auger was sprayed with 75% ethanol for disinfection firstly, and then soil samples were collected by sterilized auger with the same depth of 40 cm in each treatment plot. From each plot, soil samples were collected from 12 random sites and mixed well. These soil samples were collected in sterile plastic bags and placed on ice in an ice box. The samples were immediately transferred to the laboratory, where they were sieved through a 2-mm mesh stainless steel sieve, and then stored in a refrigerator at 4 °C for immediate analysis or were stored at -80 °C for later use. Meanwhile, portions of the soil samples were air dried

for soil chemical analyses. The sample soils had an average pH of 6.2, while the organic matter, total nitrogen, available phosphorus and potassium contents were 23.3 g kg^{-1}, 1.77 g kg^{-1}, 12.4 mg kg^{-1} and 66.1 mg kg^{-1}, respectively.

2.2.1 Soil Physical and Chemical Properties Analysis

Soil pH was measured using a pH meter (soil water ratio 1:2.5) (Reijonen et al., 2016). Soil organic matter was determined by potassium dichromate-sulfate colorimetric method (Walkley, 1935). Total nitrogen was determined by the Kjeldahl method (Tsiknia et al., 2014). Available phosphorus, and available potassium were subjected to the double acid method and flame photometry respectively (Bao, 2013).

2.2.2 Soil Microbial Numbers

Microbial numbers were determined using the agar plate dilution method modified with cycloheximide (100 μg L^{-1}) as described by Martin (1950). Rose Bengal-streptomycin agar medium and starch casein medium were used to determine the fungi and actinomycetes numbers in fresh soil samples as described by Miyashita (1997). The pH levels of the media were adjusted to 6.8 with HCl or NaOH. Microbial counts were determined for 5 replicates.

2.2.3 Soil Microbial Biomass

The soil microbial biomass N (MBN) and soil microbial biomass C (MBC) contents were determined using the chloroform fumigation-extraction method as described by Brookes et al. (1985) and Vance et al. (1987). The contents of soil microbial biomass P (MBP) contents were determined by the phosphorus molybdenum blue colorimetric method (Powlson et al., 1987).

2.2.4 Soil Enzyme Activities

β-Glucosidase (EC.3.2.1.21) assays were based on *ρ*-nitrophenol (*p*NP) release after cleavage of a synthetic substrate (Hayano, 1973). In brief, the color of the released *ρ*-nitrophenol was measured at 400 nm using a spectrophotometer (UV-1700, Shimadzu, Japan). A standard curve was plotted using 0-80 μg mL^{-1} concentrations of *ρ*-nitrophenol. Enzyme activities are expressed as nmol *p*NP released per g dry soil per minute (nmol *p*NP g^{-1} min^{-1}).

Acid phosphatase activity in soils was estimated by measuring the amount of *ρ*NP released after incubating the samples with *ρ*-nitrophenyl-phosphate (Alef et al., 1995). In a reaction tube, 0.25 mL of toluene, 4.0 mL of modified universal buffer (5x MUB, pH 6.0, which was made by dissolving 12.1 g of Tris, 11.6 g of maleic acid, 14.0 g of citric acid and 6.3 g of boric acid in 500 mL of 1 M NaOH to make a volume of 1 L), and 1.0 mL *ρ*-nitrophenyl-phosphate (15 mmol L^{-1}) were added to 1.0 g of soil sample and incubated at 37 °C for 1 h. The reaction was terminated by adding 1.0 mL of 0.5 mol $CaCl_2$ and 4.0 mL of 0.5 mol NaOH to the mixture prior to filtration. The absorbance of the released *ρ*NP was measured at 400 nm using a spectrophotometer (UV-1700, Shimadzu, Japan), and the phosphatase activity is expressed in mg *ρ*-NP g^{-1} h^{-1}.

Aminopeptidase activity was measured using the method described by Pansombat et al. (1997) with 0.002 M *N*-benzoyl-Lxycarbonylglycyl L-phenylalanine (ZGP). The absorbance at a wavelength of 570 nm was measured using a spectrophotometer (UV-1700, Shimadzu, Japan). All analyses were conducted with 5 replicates.

2.3 Analysis of Soil Microbial Diversity

Microbial community genomic DNA was extracted from samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's instructions. The DNA extract was checked on a 1% agarose gel, and the DNA concentrations and purity were determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). PCR amplification and sequencing of the total DNA extracted from the rhizosphere soil samples were performed by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China), while PCR amplification was performed using an ABI GeneAmp 9700 instrument (ABI, USA), and the PCR products were recovered using 2% agar-gel electrophoresis. The products were purified by using an AxyPrep DNA Gel Extraction Kit (Axygen, USA) and quantified using a Quantus Fluorometer (Promega, USA). The purified amplicons were pooled in equimolar quantities and were paired-end sequenced (2×300) on the Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols of the Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Raw reads were deposited in the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP284471).

2.4 Statistical Analyses

The experimental data were analyzed using Excel 2019 and Statistical Product and Service Solutions (SPSS) Statistics 21, and the results are shown as means with their standard deviations (mean±SD). Online data analysis

was conducted using the free online platform of the Majorbio Cloud Platform (http://www.majorbio.com) of the Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

3. Results

3.1 Soil Enzyme Activities

The activities of soil *β*-glucosidase in the treatments using sugarcane-soybean and sugarcane-mung bean intercropping were significantly higher than those in the monoculture and sugarcane/peanut intercropping treatment (Table 1). No significant difference in soil *β*-glucosidase activity was observed between sugarcane-peanut intercropping and the monoculture. The highest *β*-glucosidase activity was found in the sugarcane-mung bean system, which was significantly greater than that in sugarcane-soybean treatment (Table 1). Acid phosphatase activity showed nearly the same trend as that of *β*-glucosidase except that there were no significant differences between the sugarcane-soybean and sugarcane-mung bean intercropping treatments. Aminopeptidase activity was significantly different among all treatments, with the sugarcane-peanut system showing slightly lower activity than that of the monoculture (Table 1).

Table 1. Soil enzyme activities (nmol g^{-1} min^{-1} at 30 °C) in the sugarcane monoculture and different sugarcane-legume intercropping systems

Treatments	*β*-Glucosidase	Aminopeptidase	Phosphatase
Sugarcane-soybean	1.21±0.21b	10.15±0.54 b	1.58±0.09 a
Sugarcane-mung bean	1.39±0.41 a	11.32±0.27 a	1.62±0.14 a
Sugarcane-peanut	0.97±0.08 c	8.77±0.21 d	1.15±0.09 b
Monoculture	1.01±0.22 c	9.87±0.41 c	1.14±0.09 b

Note. All data are presented as means±SD (standard deviation). Different letters in the same column indicate significant differences among treatments at $P < 0.05$.

3.2 Soil Microbial Biomass

As shown in Table 2, the soil microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) contents were highest in the sugarcane-mung bean intercropping treatments. All of these three parameters were significantly higher in the sugarcane-soybean and sugarcane-mung bean intercropping systems than those of the sugarcane-peanut and monoculture treatments. The soil microbial biomass C content in the sugarcane-peanut treatment was significantly lower than that in the monoculture, but the opposite trend was observed for MBP (Table 2). The soil microbial biomass N contents in the sugarcane-peanut and monoculture treatments remained similar with those of MBN.

Table 2. Soil microbial biomass carbon (MBC), nitrogen (MBN) and phosphorus (MBP) (mg kg^{-1}) in the sugarcane monoculture and different sugarcane-legume intercropping systems

Treatments	MBC	MBN	MBP
Sugarcane-soybean	161.5±7.84 b	14.8±0.41 b	227.8±5.46 b
Sugarcane-mung bean	184.2±6.55 a	18.7±0.89 a	255.6±9.63 a
Sugarcane-peanut	111.9±5.63 d	13.4±0.56 c	199.3±4.53 c
Monoculture	137.7±9.05 c	13.5± 0.36 c	180.4 ±3.47 d

Note. All data are presented as means±SD (standard deviation). Different letters in the same column indicate significant differences among treatments at $P < 0.05$.

3.3 Soil Cultivable Microorganisms

The relative numbers of cultivable bacteria, fungi and actinomycetes in the soils of the sugarcane-soybean, sugarcane-mung bean and sugarcane-peanut treatments and the sugarcane monoculture followed a somewhat similar pattern, as the MBC, MBN and MBP contents (Table 3). In particular, the sugarcane-soybean and sugarcane-mung-bean systems were superior to the other two treatments. Notably, the abundances of cultivable fungi and actinomycetes in the sugarcane-peanut and sugarcane monoculture systems did not show any

significant variations, but the cultivable bacterial population was lower in the sugarcane-peanut intercropped soil than those in the monoculture (Table 3).

Table 3. Soil enzyme activities (nmol g^{-1} min^{-1} at 30 °C) in the sugarcane monoculture and different sugarcane-legume intercropping systems

Treatment	Bacteria (10^6 CFU·g^{-1})	Fungi (10^4 CFU·g^{-1})	Actinomycetes (10^6 CFU·g^{-1})
Sugarcane-soybean	24.31±0.16 b	5.84±0.58 b	24.87±0.66 b
Sugarcane-mung bean	29.92±0.22 a	6.14±0.67 a	27.75±1.22 a
Sugarcane-peanut	15.96±0.47 d	4.24±0.64 c	15.89±0.74 c
Monoculture (CK)	18.54±0.57 c	4.49±0.23 c	15.54±0.85 c

Note. All data are presented as means±SD (standard deviation). Different letters in the same column indicate significant differences among treatments at $P < 0.05$.

3.4 Soil Bacterial Diversity and Richness

The Shannon index, which describes bacterial diversity, was highest for the soil from sugarcane-mung bean intercropping compared to the other three treatments (Table 4). For the other parameters described so far, the bacterial diversities of the soils from the sugarcane-soybean and sugarcane-mung bean intercrops were significantly higher than those of the sugarcane-peanut and sugarcane monoculture systems. In addition, the bacterial richness indices, such as Ace and Chao1, showed that sugarcane-mung bean and sugarcane-soybean did not differ in bacterial richness but were richer than those of other treatments. Our data indicate increased bacterial abundance in the sugarcane-peanut treatment compared to the sugarcane monoculture (Table 4).

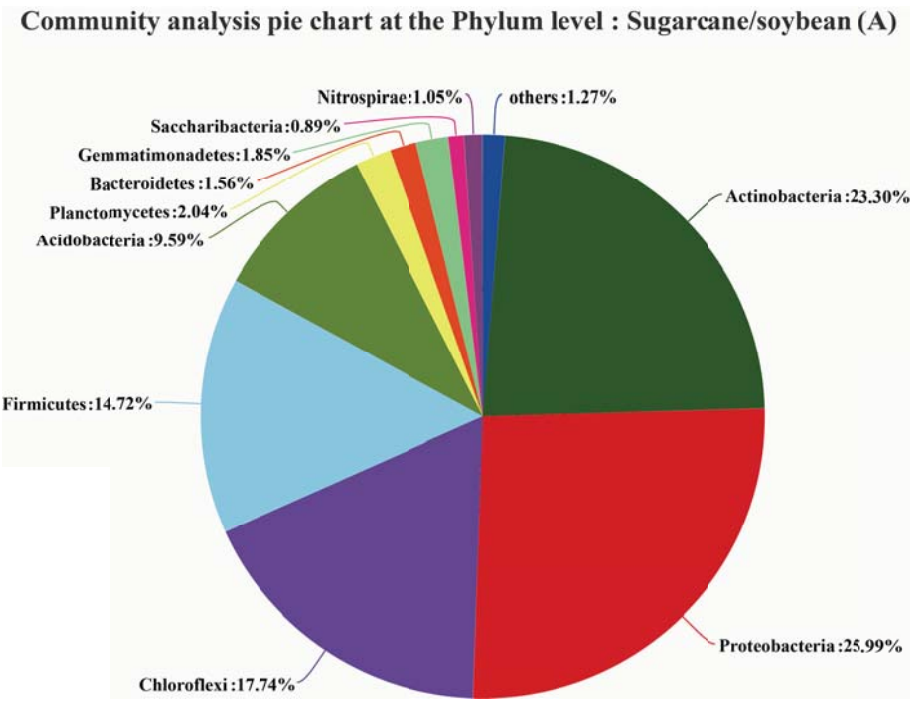
Table 4. Richness and diversity at a similarity level of 97% for soil bacteria in the sugarcane monoculture and different sugarcane-legume intercropping systems

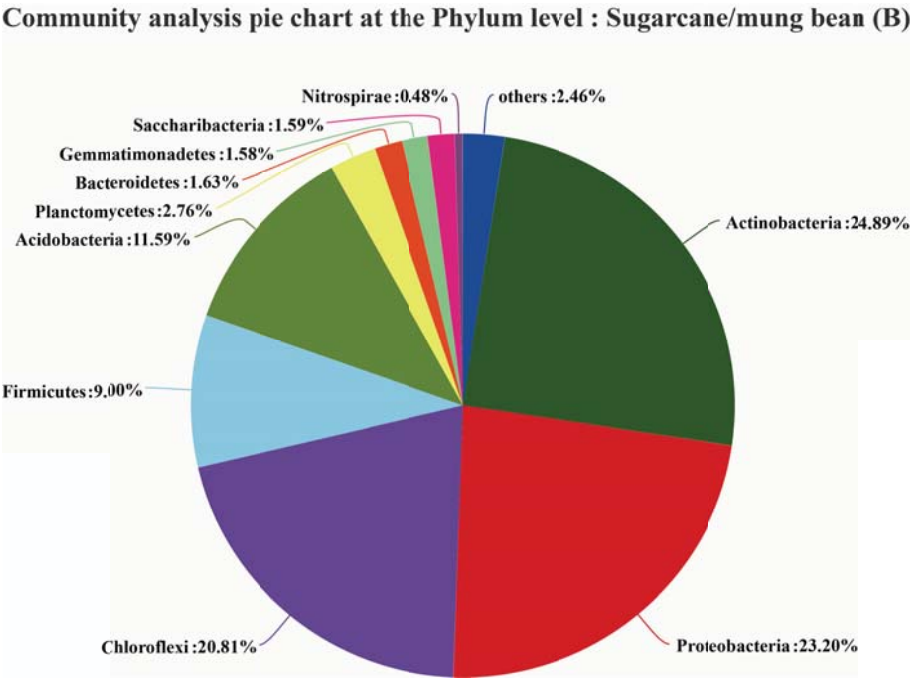
Treatments	Shannon index	Ace index	Chao1 index	Coverage
Sugarcane-soybean	6.15±0.07ab	2395.2±20.2a	2411.4±36.0a	0.98
Sugarcane-mung bean	6.34±0.02a	2429.6±114.4a	2453.2±95.7a	0.98
Sugarcane-peanut	5.92±0.23b	1860.2±83.0b	1892.3±52.7b	0.99
Monoculture	5.95±0.04b	1639.6±22.5c	1683.9±17.3c	0.99

Note. All data are presented as means±SD (standard deviation). Different letters in the same column indicate significant differences among treatments at $P < 0.05$.

3.5 Compositions of Soil Bacterial Communities in the Sugarcane Monoculture and Sugarcane-Legume Intercropping Systems

At the phylum level, the dominant soil bacteria (*i.e.*, relative abundances greater than 1%) in fields of sugarcane intercropping or monoculture systems can be divided into ten (sugarcane-soybean and sugarcane-mung bean intercrops), nine (sugarcane monoculture) and eight (sugarcane-peanut intercrop) phyla (Figure 1). In the sugarcane monoculture, the proportions of the dominant bacterial groups (ordered from large to small) were Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Firmicutes, Planctomycetes, Bacteroidetes, Gemmatimonadetes and the others group. Their relative proportions were 25.05%, 22.20%, 21.53%, 11.49%, 11.45%, 2.68%, 1.28%, 1.06%, and 1.95%, respectively. In contrast, the relative abundances (shown in parentheses) of the dominant bacteria in the sugarcane-soybean treatment (Figure 1A) were Proteobacteria (25.99%), Actinobacteria (23.30%), Chloroflexi (17.74%), Firmicutes (14.72%), Acidobacteria (9.59%), Planctomycetes (2.04%), Gemmatimonadetes (1.85%), Bacteroidetes (1.56%), Nitrospirae (1.05%) and others (1.27%). In the sugarcane-mung bean treatment (Figure 1B), the compositions and abundances (shown in parentheses) of the dominant bacteria were Actinobacteria (24.89%), Proteobacteria (23.20%), Chloroflexi (20.81%), Acidobacteria (11.59%), Firmicutes (9.00%), Planctomycetes (2.76%), Bacteroidetes (1.63%), Saccharibacteria (1.59%), Gemmatimonadetes (1.58%) and others (2.46%). In the sugarcane-peanut treatment, Actinobacteria (23.53%), Proteobacteria (21.12%), Chloroflexi (22.49%), Firmicutes (14.21%), Acidobacteria (10.67%), Planctomycetes (3.11%), and Saccharibacteria (1.40%) dominated, and their relative proportions are shown in parentheses (Figure 1C).


Community analysis pie chart at the Phylum level : Sugarcane/soybean (A)
Nitrospirae:1.05%
others:1.27%
Saccharibacteria:0.89%
Gemmatimonadetes:1.85%
Bacteroidetes:1.56%
Planctomycetes:2.04%
Acidobacteria:9.59%
Actinobacteria:23.30%
Firmicutes:14.72%
Proteobacteria:25.99%
Chloroflexi:17.74%


Community analysis pie chart at the Phylum level : Sugarcane/mung bean (B)
Nitrospirae:0.48%
others:2.46%
Saccharibacteria:1.59%
Gemmatimonadetes:1.58%
Bacteroidetes:1.63%
Planctomycetes:2.76%
Acidobacteria:11.59%
Actinobacteria:24.89%
Firmicutes:9.00%
Chloroflexi:20.81%
Proteobacteria:23.20%

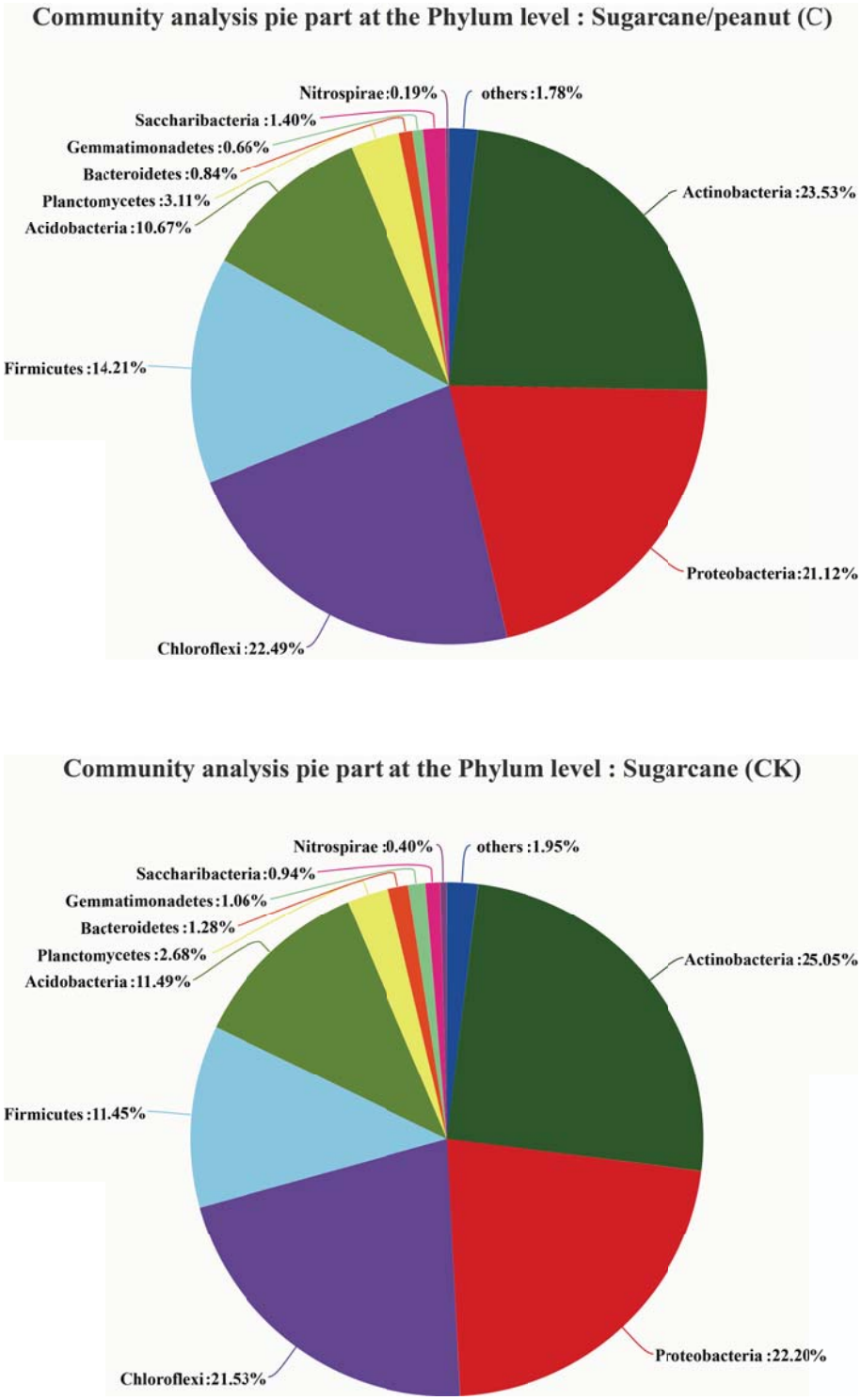


Figure 1. Compositions of soil bacterial communities at the phylum level among the sugarcane monoculture and sugarcane-legume intercrops

Note. A: sugarcane intercropped with soybean, B: sugarcane intercropped with mung bean, C: sugarcane intercropped with peanut, and CK: sugarcane monoculture.

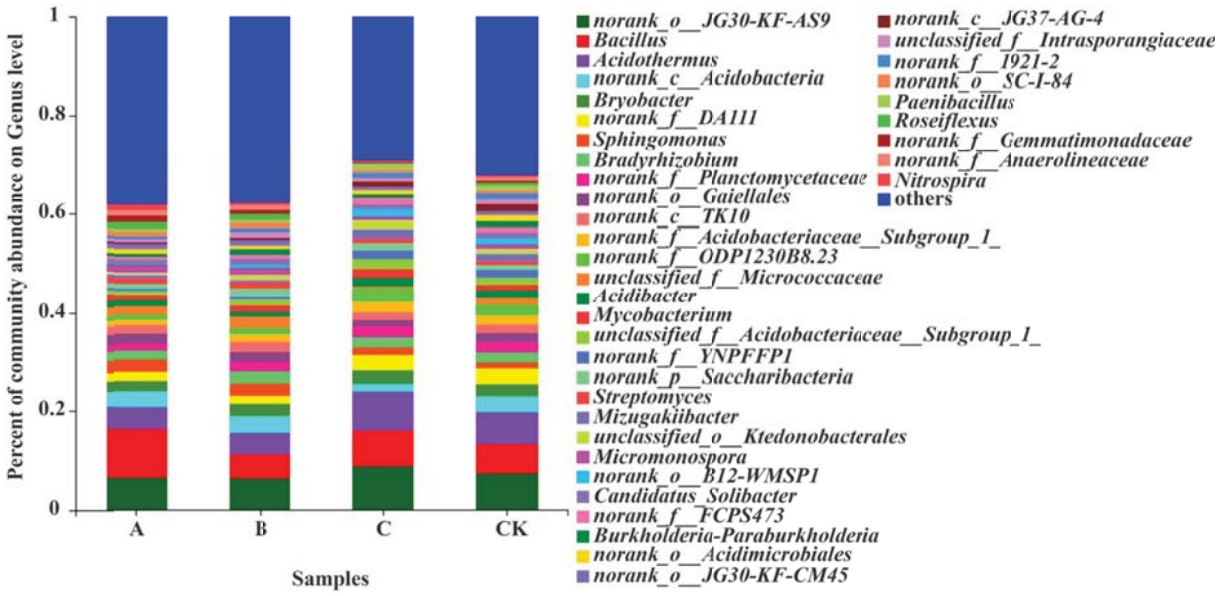


Figure 2. Compositions of soil bacterial communities at the genus level among the sugarcane monoculture and sugarcane-legume intercrops

Note. A: sugarcane intercropped with soybean, B: sugarcane intercropped with mung bean, C: sugarcane intercropped with peanut, and CK: sugarcane monoculture.

Figure 2 shows that the dominant soil bacteria in sugarcane fields under the four treatments were consisted of over 39 genera. Table 5 lists the compositions and relative proportions of the dominant soil bacterial genera that were identified in this study. The numbers of identified bacterial genera in the A, B, C and CK treatments were 24, 28, 26 and 27, respectively.

All of these results show that the intercropping treatments not only changed the proportions of dominant soil bacteria but also altered the compositions and structures of soil bacterial communities (Table 5).

Table 5. Compositions of dominant soil bacterial communities at the genus level and their relative abundances (%) in soils from the sugarcane monoculture and sugarcane-legume intercrops

Genus	A	B	C	CK
norank_o__JG30-KF-AS9	6.40	6.26	8.71	7.26
Bacillus	9.96	4.87	7.31	5.97
Acidothermus	4.48	4.47	7.94	6.55
norank_c__Acidobacteria	3.21	3.44	1.58	3.25
Bryobacter	2.05	2.45	2.56	2.41
norank_f__DA111	1.66	1.57	3.07	3.02
Sphingomonas	2.58	2.54	1.61	1.28
Bradyrhizobium	1.74	2.25	1.95	1.96
norank_f__Planctomycetaceae	1.44	1.97	2.30	2.13
norank_o__Gaiellales	2.05	2.02	1.30	1.81
norank_c__TK10	1.71	2.05	1.53	1.72
*norank_f__Acidobacteriaceae__Subgroup_1_*	1.13	1.59	2.26	1.95
norank_f__ODP1230B8.23	-	1.06	2.68	2.06
unclassified_f__Micrococcaceae	1.84	2.47	-	1.40
Acidibacter	1.29	1.02	1.82	1.74
Mycobacterium	1.19	1.30	1.82	1.15
*unclassified_f__Acidobacteriaceae__Subgroup_1_*	-	1.16	1.95	1.42
norank_f__YNPFFP1	-	-	1.84	1.65
norank_p__Saccharibacteria	-	1.57	1.41	-
Streptomyces	1.26	1.37	-	-
Mizugakiibacter	-	-	1.87	1.47
unclassified_o__Ktedonobacterales	-	1.06	1.74	-
Micromonospora	1.51	1.35	-	-
norank_o__B12-WMSP1	-	-	1.64	1.39
Candidatus_Solibacter	-	1.21	-	1.06
norank_f__FCPS473	-	-	1.32	1.07
Burkholderia-Paraburkholderia	-	1.17	-	1.17
norank_o__Acidimicrobiales	-	-	-	1.21
norank_o__JG30-KF-CM45	1.01	1.11	-	-
norank_c__JG37-AG-4	-	-	1.09	1.47
unclassified_f__Intrasporangiaceae	-	1.16	-	-
norank_f__1921-2	-	-	1.02	1.15
norank_o__SC-I-84	1.00	1.08	-	-
Paenibacillus	-	-	1.26	-
Roseiflexus	1.30	1.17	-	-
norank_f__Gemmatimonadaceae	1.32	-	-	-
norank_f__Anaerolineaceae	1.07	-	-	-
Nitrospira	1.19	-	-	-
others	38.13	37.8	29.29	32.26

Note. A: sugarcane intercropped with soybean, B: sugarcane intercropped with mung bean, C: sugarcane intercropped with peanut, and CK: sugarcane monoculture.

The total numbers of bacteria in the sugarcane-soybean, sugarcane-mung bean, sugarcane-peanut and monoculture soil samples were 505, 492, 403 and 464, respectively (Figure 3-1: A, B, C, and CK). Additionally, the numbers of unique bacteria in these treatments (in the same order) were 28, 22, 0 and 2, respectively (Figure 3-1A, B, C, CK). The total numbers of bacteria at the operational taxonomic units (OTUs) level in the sugarcane-soybean sugarcane-mung bean, sugarcane-peanut (C) and sugarcane monoculture treatments were 2,609, 2,553, 1,910 and 2,358, respectively (Figure 3-2: A, B, C, CK). The unique bacteria numbers in these respective treatments were 214, 118, 23 and 49 (Figure 3-2: A, B, C, CK). These results indicate that the soil

bacterial community structures of sugarcane soils can be significantly altered by legume intercropping, particularly with soybean and mung bean.

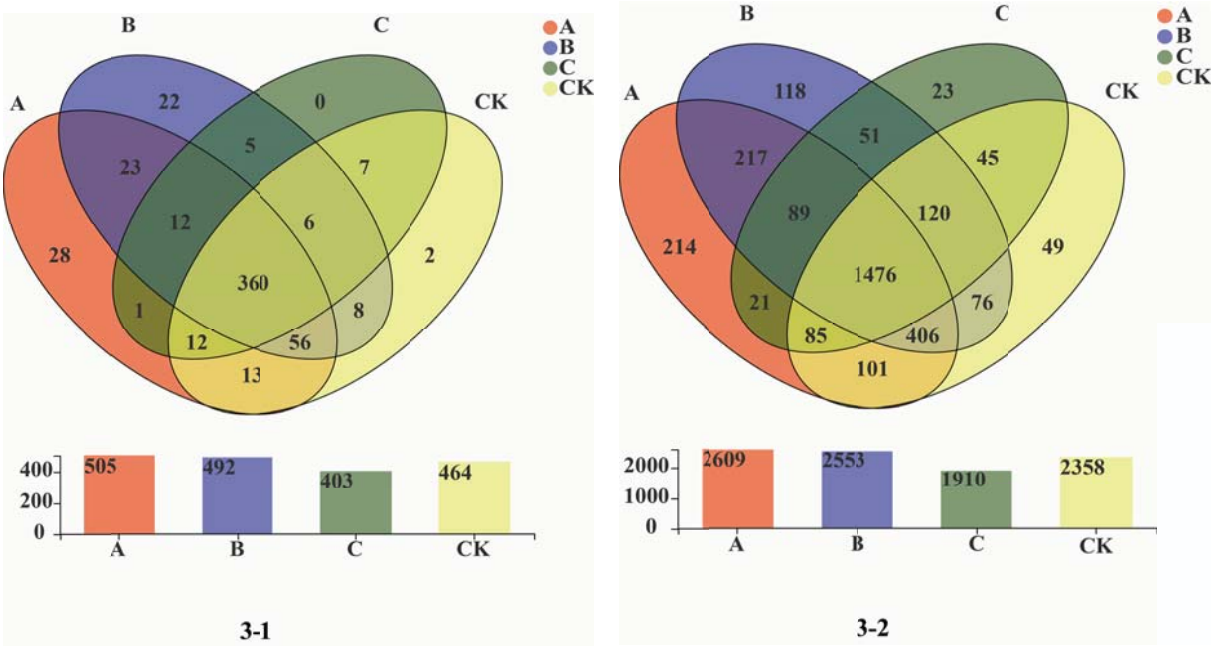


Figure 3. Venn analyses of soil bacteria for the four intercropping treatments at the genus (3-1) and OTU (3-2) levels

Note. A: sugarcane intercropped with soybean, B: sugarcane intercropped with mung bean, C: sugarcane intercropped with peanut, and CK: sugarcane monoculture.

4. Discussion

Previous studies have shown that intercropping is more beneficial to soil nutrient enrichment, plant nutrient acquisition and productivity than the corresponding monocultures. For example, researchers in China and sub-Saharan Africa have observed better crop yields and resource use even under adverse production conditions by using cereal-legume intercropping than monocultures (Kumar et al., 1998; Zhang & Li, 2003; Mucheru-Muna et al., 2010; Wang et al., 2014). Despite these outcomes and the fact that legumes are known to contribute plant available nitrogen and boost yields and soil health, a mechanistic understanding of the intercropping-dependent improvement of soil health and crop yield remains poorly understood.

As mentioned before, low soil fertility is a critical limiting factor for cane yields and quality in China (Zeng et al., 2015). Soil acidification and all accompanying soil biotic and abiotic constraints for sugarcane crop improvement are widespread in many sugar-producing regions in China. In this context, legume intercropping with sugarcane appears to be a very desirable crop management strategy for reversing soil degradation and improving soil fertility and crop productivity, as has been observed for other broad acre crops (Zhang & Li, 2003; Mucheru-Muna et al., 2010; Wang et al., 2014). Collectively, the results obtained in this study also support this assertion. For instance, soil microbial biomass is an important indicator of soil quality, soil fertility and crop productivity (Powlson et al., 1987). The greater the microbial biomass in soil, the greater the capacity of the soil to provide plant nutrients by mineralization of organic nutrients (Dwivedi & Soni, 2011). Soil microbial biomass carbon promotes the formation of new humus and increases the soil total carbon content (Doran et al., 1996). Similarly, soil microbial biomass nitrogen reflects the availability of soil nitrogen to crops and plays an important role in soil nitrogen turnover and supply (Doran et al., 1996). Additionally, turnover of soil microbial biomass phosphorus, although it is not directly available to plants, releases inorganic phosphorus, which is very important for plant growth (Khan & Joergensen, 2009). In this study, soil microbial biomass carbon, nitrogen and phosphorous were significantly higher in the sugarcane-mung bean and sugarcane-soybean intercropping treatments than in sugarcane-peanut treatments and sugarcane monoculture. These results suggest that soil

microbial biomass in sugarcane fields can be improved considerably by intercropping with soybean, mung bean or, with a much reduced effect, peanut.

Soil enzymes are produced by microorganisms, other soil organisms and plant roots, and they have key biochemical functions, such as decomposing organic matter in the soil system (Ellert et al., 1997), and thereby release nutrients that are readily available for crop uptake. Soil enzymes also play an important role in facilitating microbial processes in the soil that stabilize soil structure, balance soil microbial ecology and drive nutrient cycling (Dick et al., 1994). In our study, the activities of soil β-glucosidase, aminopeptidase and acid phosphatase in sugarcane-soybean and sugarcane-mung bean intercropping systems were significantly higher than in sugarcane monocultures. This finding parallels the soil microbial biomass levels observed in our study, which indicate a significant contribution to nutrient cycling that is facilitated by increased levels of soil microflora in legume-intercropped sugarcane crops. Intercropping with soybean or mung bean thus significantly accelerates soil carbon, nitrogen and phosphorus cycles in sugarcane fields and promotes soil fertility and healthy soil ecology, which in turn, result in better crop performance

Soil microbial populations and their compositions are closely related to soil quality, which make them an ideal indicator of soil health (Brookes, 1995; Zhang et al., 2014). In our study, we found that the soil microbial population in sugarcane monocultures can be significantly improved by intercropping with soybean and mung bean but not as effectively with peanut. High-throughput sequencing of microbial populations revealed that the dominant bacteria at the phylum level represented eight phyla in sugarcane monocultures, *e.g.*, Actinobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Firmicutes, Planctomycetes, Bacteroidetes, and Gemmatimonadetes groups. In addition to these eight bacterial phyla in sugarcane monoculture fields, sugarcane-soybean soils were additionally enriched with Nitrospirae, while Saccharibacteria formed another dominant phylum in sugarcane-mung bean intercropped soils. Additionally, it is noteworthy that Saccharibacteria was also enriched in sugarcane-peanut intercropped fields. In addition to the new added dominant phyla, the orders of the dominant bacteria, based on their abundance levels, were also changed in intercropped soils. For instance, Proteobacteria was the third dominant phylum in sugarcane-peanut treatments but was first and second most abundant in sugarcane-soybean and sugarcane-mung bean systems, respectively. Generally, Proteobacteria are considered to be copiotrophic microorganisms, which thrive under conditions of high nutrient availability (Chen et al., 2016). These results suggest that legume intercropping promotes nutrient-tolerant soil bacterial community structures in sugarcane fields and thus positively impacts soil fertility. As the microbial community structures in rhizospheres can be changed by one or synergistically by both plant species (Yang et al., 2016; Song et al., 2007a, 2007b), we found significant variations in bacterial community compositions and abundances in intercropped soils compared to monoculture treatments. Collectively, our data suggest that intercropping systems, such as sugarcane-soybean and sugarcane-mung bean, are beneficial for improving soil biology and ecology, soil structure and soil fertility and lead to superior sustainable sugarcane agriculture. It is very likely that sugarcane-legume intercropping will also help meet the increasing demand for agricultural intensification and diversification without compromising the environmental obligations and economic outcomes of sugarcane agriculture.

5. Conclusion

In this study, a field experiment was carried out to elucidate the effects of intercropping sugarcane with different legumes on soil biological properties, soil bacterial diversities and community structures. The conclusions are as follows: The biological indicators of soil fertility in sugarcane fields, such as the activities of soil cultivable microorganisms (*e.g.*, bacteria, fungi and actinomycetes), soil enzymes (*e.g.*, β-Glucosidase, acid phosphatase, and aminopeptidase) and microbial biomass carbon, nitrogen, phosphorus, were all significantly improved by intercropping sugarcane with soybean and mung bean. Soil bacterial diversity and richness in sugarcane fields were also significantly enhanced by sugarcane intercropping with soybean and mung bean. By intercropping, new bacterial phyla, such as Nitrospirae or Saccharibacteria, became the dominant groups in intercropped soils. Proteobacteria, which thrives under conditions of high nutrient availability, became the most and second-most dominant bacterial group in sugarcane-soybean and sugarcane-mung bean systems. Sugarcane-mung bean intercropping showed the greatest effects for improving soil fertility and soil health among the cropping systems studied in this work.

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Form of Distribution of Dendro/Morphometric Variables for Brazilian Pine in Southern Brazil

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Abstract

The form of distribution found for the dendro/morphometric variables determines the structure, stability, productivity of forest stands, being a tool to propose silvicultural interventions, management, conservation of species, and dynamics of this environment. Thus, this study evaluates, using probability density functions (pdf), the form of distribution of these variables for araucaria in five sites in southern Brazil, aiming to establish the dynamics and identify the existence of a standard—or the lack thereof—to propose the need for silvicultural interventions to conserve the species and the future forest structure. The Normal, Log-Normal, Weibull and Gamma probability density functions were tested. Results show no significant changes in the shape and dimension in the forest structure dynamics, but a period of stability in the pattern of dendro/morphometric values, resulting from the stagnation of the values of the variables, non-intervention in the forest, relationship with the site, density, competition, and position of the tree in the forest stratum, which compromises the future structure of this forest typology. The study proves that the distribution probability of the variables can be used in management for species conservation and future structure development, as this influences the growth dynamics and processes, resource availability, and the stability, diversity, vitality, and productivity of the species.

Keywords: *Araucaria* angustifolia, degree of slenderness, crown ratio, crown diameter

1. Introduction

As mixed species forests are advancing, forest science should provide forestry management with appropriate methods for establishing and regulating mixed species stands. Mixed species stands fundamentally differ from this, with trees exhibiting their full inter and intra-specific structural variability and plasticity. Their traits were probably developed by co-evolution in natural mixed species stands, but became less visible and important in artificial monocultures (Pretzsch, 2019). According to the same author, the structure and size of tree crowns are highly relevant for a tree's fitness. They determine the tree's access to resources, the availability and occupation of space, size growth, and seed production and dispersal. In fully stocked stands, crown size growth results in competition for space, leading to social differentiation, growth reduction of suppressed trees, mortality, and self-thinning (White et al., 2007).

The structure and size of the crown are also practically and economically relevant. Wide crowns mean high mechanical stability (Knoke et al., 2008), due to low slenderness ratio (hd), but low wood quality, due to the number and thickness of branches (Pretzsch et al., 2016). Many recent studies show that in mixed species stands, trees can have wider and longer crowns (Bayer et al., 2013; Barbeito et al., 2017; Olivier et al., 2016) and higher mechanical stability (Pretzsch, 2019), but also inferior wood quality (Pretzsch et al., 2016). In mono-specific stands, wider crowns are associated with more rigorous self-thinning and lower tree numbers per unit area; in mixed stands, in turn, the wider crown can be coupled with higher stand density.

From a forest management perspective, it is necessary to understand how the morphometric characteristics affect the forest's present and future productivity and structure. Studies on the morphometry of forest species aim to reconstruct the space occupied by the individual tree, assess the degree of competition in a stand, identifying the stability, vitality, and productivity of each tree (Costa et al., 2016; Hess et al., 2016; Hess et al., 2018b; Klein et al., 2017; Minatti et al., 2016; Roman et al., 2009).

Despite the several studies on araucaria morphometry, information regarding the form of distribution of dendro/morphometric variables is still insufficient, especially in forests subjected to a non-management regime, as in the Mixed Ombrophilous Forests in southern Brazil. Such information is of great importance, since the analysis of the form of distribution of dendro/morphometric variables works as a reference for the control of forest density, species composition, growth of individual trees, regeneration of understory, genetic diversity, structural conditions of future forests, intervention planning, and balance in the distribution of age and diameter classes.

Adjusting probability density functions (pdf) to assess the distribution of dendro/morphometric variables is an important tool to analyze the current situation of the forest structure at its site. Thus, aiming at the conservation of araucaria species, the multiple use and maintenance of mixed rainforest ecosystem, our hypotheses are: (1) the distribution of dendro/morphometric variables, their pattern or lack thereof, is related to the absence of forest management; (2) the pattern of morphometric indices is related to and influences the structure and dynamics of the forest; and (3) the probabilistic behavior of the distribution may indicate the need for silvicultural intervention.

This study aimed, thus, to adjust the probability density functions to establish the pattern of dendro/morphometric variables, or its absence, for *Araucaria angustifolia* (Bertol.) Kunzte, allowing us to obtain information and knowledge to assist in silvicultural interventions, management plans, and conservation of the future structure of araucaria forests.

2. Material and Methods

2.1 Study Areas and Data Measurement

The sites sampled are remnants of Mixed Ombrophilous Forest (MOF) with a natural occurrence of *Araucaria angustifolia*, located in the state of Santa Catarina (Figure 1). The sampled trees were grouped by site, totaling five site samples, each differing in the number of samples and individuals and sampling process (Table 1); this does not interfere in the result analysis, since our goal is to analyze dendro/morphometric variables per individual.

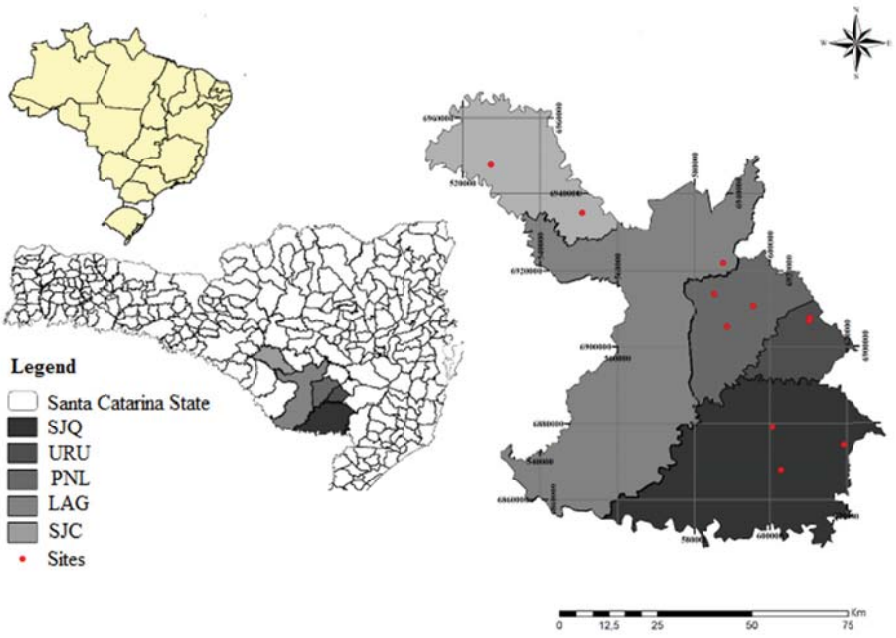

Figure 1. Map indicating the sites for measuring dendro/morphometric variables for araucaria in Santa Catarina, Southern Brazil

The region encompassing the five sites is characterized by Cfb climate according to the Köppen classification: temperate climate, constantly wet and without a dry season. In São Joaquim (SJQ), the altitude is 1,352 m, with 14 °C average annual temperature and 1,683 mm precipitation. In Urupema (URU), the altitude is 1,324 m, with 13.7 °C average temperature and 1,722 mm precipitation. In Painel (PNL), the altitude is 1,123 m, with 15.3 °C average annual temperature and 1,543 mm precipitation. In Lages (LAG), the altitude is 987 m, with 15.2 °C average temperature and 1,685 mm precipitation. In São José do Cerrito (SJC), the altitude is 876 m, with 16.7 °C average temperature and 1,570 mm precipitation (Alvares et al., 2013).

Table 1. Number of samples, sampling process per site and number of individual trees sampled from araucaria in the remnants of mixed ombrophilous forest in southern Brazil

Number of Samples	Site	N	Sampling process	Author
1	SJQ	63	Individual tree	Hess et al. (2016) *
2		53	Individual tree	Hess et al. (2016)
3		70	Individual tree	Minatti et al. (2016) *
4	PNL	62	Individual tree	Hess et al. (2016)
5		127	SAC	Ricken (2018) *
6		70	Individual tree	Minatti et al. (2016)
7	URU	61	Individual tree	Hess et al. (2016)
8		70	Individual tree	Minatti et al. (2016)
9	LAG	332	Fixed parcel	Silveira et al. (2018) *
10	SJC	77	SAC	Ricken (2018) *
11		127	Individual tree	Klein et al. (2017) *
Total		1.111		

Note. SJQ, PNL, URU, LAG, SJC: study sites São Joaquim, Painel, Urupema, Lages, São José do Cerrito; N: number of trees; * authors and studies cited in the references; SAC: sample by angular count Bitterlich method.

All trees with diameter at breast height greater than or equal to 10 cm had their diameter, total height and four crown rays in the north, south, east, and west cardinal directions measured with a compass and a TruPulse hypsometer. From these data, we calculated the following morphometric indices:

$$hd = h/d \quad (1)$$

$$cd = 2 \times \overline{cmr} \quad (2)$$

$$cr = \frac{cl}{h} \times 100 \quad (3)$$

where, hd: degree of slenderness; h: height in m; d: diameter at breast height in cm; cd: crown diameter in m; $\overline{cmr}$: crown mean radius; cr: crown ratio in %; cl: crown length.

2.2 Data Analysis

For each site, the data were classified by diametric class and respective morphometric value. Evaluation was based on the histogram of the frequency distribution of the diametric distribution and morphometric variables degree of slenderness (hd), crown ratio (cr) and crown diameter (cd). Sturges' rule was used to determine the number of classes, and the interval between classes was obtained by the ratio of the total amplitude to the number of classes.

2.3 Adjustment and Evaluation of Probability Density Functions (PDF) for Dendro/Morphometric Variables

The form of distribution of the variables was evaluated by adjusting probability density functions, then tested for normal, log-normal, Weibull, exponential and gamma distribution functions (Table 2). Distribution parameters were estimated using the maximum likelihood method. All adjustments were made using the PROC CAPABILITY procedure from the SAS statistical package (SAS Institute, 2011).

Table 2. Probability density functions tested to adjust diameter distribution and morphometric indices of *Araucaria angustifolia* at different sites in southern Brazil

Function	Formula	Conditions
N[a]	$f(X;\alpha,\beta) = \frac{1}{\beta\sqrt{2\pi}} \exp\left[\frac{(x-\alpha)^2}{2\beta^2}\right]$	α = population average β = population standard deviation
LN[b]	$f(X;\alpha,\beta,m) = \frac{e^{-\frac{[\ln(x-\alpha)]^2}{m}}}{(x-\alpha)\beta\sqrt{2\pi}}$	α = location parameter β = shape parameter m = scale parameter
G[c]	$f(x) = \frac{1}{[\beta^{\alpha}\gamma(\alpha)] + (x-\varepsilon)^{\alpha-1}} .\exp\left[-\frac{(x-\varepsilon)}{\beta}\right]$	α = location parameter β = scale parameter ($\beta > 0$) ε = lowest observed value
W[d]	$f(x) = \left(\frac{c}{b}\right).\left(\frac{x-\alpha}{b}\right)^{c-1}.\exp\left\{-\left[\frac{(x-\alpha)^c}{b}\right]\right\}$	α = location parameter b = scale parameter c = shape parameter
E[e]	$f(x) = \begin{cases} 1 \\ \beta \end{cases} . e^{-\frac{x}{\beta}}$ for $x \geq 0, \beta > 0$	β = function parameter e = Euler number

Note. a: Meyer, 1978; b: Limpert et al., 2001; c: Schneider et al., 2009; d: Silva, 2003; N: normal; LN: log-normal; G: gamma; W: Weibull; E: Exponential.

The Anderson-Darling test was used considering a 5% probability to assess the quality of the fit. Model performance was evaluated according to the probability value associated with the statistic. Non-significant values indicate fit and significant values indicate no fit. When there was no adjustment at 5%, we considered the model that fit at 1% probability.

The Anderson-Darling test, also known as the Anderson-Darling chi-square, is a way of estimating the minimum distance and one of the most powerful statistics for detecting most forms of normality, with two main applications: 1) test the null hypothesis that a batch of data is a random sample from a normally distributed population; 2) test the goodness of the fit of a distribution (SAS Institute, 2011). The Anderson-Darling statistic (A^2) is defined by the following equation:

$$A^2 = -n - \frac{1}{n}\sum_{i=1}^{n}\left[\left(2i - 1\right)\log U_{(i)} + \left(2n + 1 - 2i\right)\log(1 - U_{(i)})\right] \quad (4)$$

where, A^2: Anderson-Darling statistic; U:F(X): transformation of the probability integral of variable X; X: morphometric variable considered; n: number of independent observations; i: observation number; log: natural logarithm.

2.4 Analysis of the Form of Distribution of Dendro/Morphometric Variables—Past and Future Characteristics

We used the frequency distribution graph and the frequency table for each variable and site to identify, analyze, and interpret the current distribution of the dendro/morphometric variables that make up the structure of individual trees in the forest. Thus, the form of distribution of the dendro/morphometric variables was identified based on their values and form of distribution generated by the probability density function. Analyzing and interpreting the form of distribution allowed us to determine the conditions of the individual trees and the forest structure, and to make inferences regarding the shape-dimension of the crown, diameter at breast height, growth conditions of the species and formation of a future structure.

3. Results

Results show that the dendro/morphometric variables at the studied sites are within a cycle of non-significant structure changes as a whole, seen in the concentration of values in a single form of distribution, showing similarities that may indicate slowly changes in the shape-dimension (Tables 3 and 4) (Figures 2, 3, 4 and 5). These similarities may appear as site characteristics, stage of forest succession, and degree of past disturbance, but occur mainly due to the lack of silvicultural intervention regimes in this forest typology over the past 25 years—causing the forest's stability and structural stagnation. Conditions that show the dominance and formation of a regular mono-species forest (araucaria), not ideal for mixed forests.

By analyzing and interpreting the RF percentage value (Table 4), can inform and identify this structural stability in terms of dimension, morphometry, canopy, and crown size. From an ecological perspective, such structural stability is extremely harmful in mixed forests, since it shows less complexity as an adaptive response to changes in the environment, diversity, and dynamics of the forest.

This interpretation finds confirmation in the d values and morphometric variables. Higher diametric concentration in the 30 to 60 cm classes shows that the forest has a lower rate of ingrowth (trees that migrate from one diameter class to another) and regeneration, while in mixed forests the correct would be an inverted J-distribution. This also indicates a compromise of a future structure with old growth trees, less diametric increase, closed canopy, less sunlight entry, lower temperature and, consequently, reduced seed dormancy break and less species diversity.

For sites presented hd ratio of 40, which indicates a higher growth in d than in h, older and slow-growing trees (Hess et al., 2020). Lower cr values influence the photosynthetic and productive capacity of the forest. For araucaria, trees with lower cr value indicate older age, trees that occupy the upper stratum, or are in extreme competition, and ontogenetic characteristics of the species (when in adequate growth conditions). Lower cd values, in turn, indicate a lack of lateral space, crown expansion, greater density, site characteristics (less soil depth, predregosity, etc.) and position of the tree in the stratum.

Table 3. Descriptive statistics for the dendro/morphometric variables of *Araucaria angustifolia* at five study sites in southern Brazil

Variable	Site	Average	Minimum	Maximum	CV%
d	SJQ	55.9	20.0	127.6	39.7
	PNL	51.2	17.8	94.2	29.0
	URU	46.3	18.8	89.4	31.0
	LAG	29.0	10.1	88.5	51.5
	SJC	37.3	11.5	97.1	38.6
hd	SJQ	37.0	14.3	77.1	35.1
	PNL	36.7	19.3	99.9	29.3
	URU	36.2	20.0	82.7	29.6
	LAG	65.8	20.8	134.7	35.9
	SJC	48.8	13.5	93.7	31.1
cr	SJQ	20.8	1.3	63.4	50.9
	PNL	27.5	2.7	72.7	52.4
	URU	37.3	2.3	66.9	33.5
	LAG	32.4	3.3	70.8	43.2
	SJC	46.1	5.3	84.8	32.0
cd	SJQ	10.4	4.2	18.3	26.3
	PNL	8.6	1.1	22.9	40.2
	URU	8.8	4.1	15.9	24.7
	LAG	5.5	0.1	14.3	53.3
	SJC	7.7	2.7	20.5	41.4

Note. d: diameter at breast height in cm; hd: degree of slenderness; cr: crown ratio in %; cd: crown diameter in m; SJQ, PNL, URU, LAG and SJC: study sites São Joaquim, Painel, Urupema, Lages and São José do Cerrito.

Table 4. Analysis results of the distribution of dendro/morphometric variables, lower and upper limit and model that identifies the form of distribution of the variables for araucaria at different sites in southern Brazil

Site	Morphometric indices	Morphometric pattern			Model (pdf)	Parameter			Prob. > A^2
		Lower limit	Upper limit	RF (%)		Location	Scale	Form	
SJQ	d	32	68	65	Weibull	20.1	39.50	1.62	0.036
	hd	21	42	62	Weibull	14.3	25.44	1.77	0.250
	cr	8	29	74	Weibull	1.3	21.88	1.89	0.078
	cd	6	14	87	Normal	10.4	2.73	-	0.250
PNL	d	33	65	72	Normal	51.2	14.86	-	0.084
	hd	28	46	71	Weibull	19.3	19.40	1.66	0.010
	cr	10	34	63	Weibull	2.7	27.79	1.75	0.250
	cd	7	11	50	Normal	8.6	3.44	-	0.029
URU	d	26	58	74	Normal	46.3	14.34	-	0.250
	hd	27	43	70	Gama	19.9	6.88	2.38	0.021
	cr	18	58	92	Normal	37.3	12.50	-	0.250
	cd	7	10	57	Weibull	4.0	5.41	2.30	0.073
LAG	d	10	59	31	Weibull	10.1	19.80	1.15	0.011
	hd	32	68	58	Gama	20.7	14.16	3.18	0.025
	cr	10	52	87	Weibull	3.3	32.66	2.13	0.010
	cd	3	6	46	Weibull	0.4	5.85	1.87	0.189
SJC	d	20	50	80	Gama	11.5	8.42	3.07	0.061
	hd	31	67	75	Weibull	13.6	10.00	3.51	0.051
	cr	32	68	87	Weibull	5.1	45.60	2.77	0.011
	cd	4	10	75	Gama	2.7	2.02	2.49	0.027
Geral	d	10	60	85	Weibull	10.1	32.10	1.63	0.010
	hd	21	54	69	Weibull	13.5	38.49	1.74	0.010
	cr	8	48	80	Weibull	1.3	35.08	2.05	0.198
	cd	2	12	85	Weibull	0.4	8.42	2.30	0.250

Note. SJQ: São Joaquim; PNL: Panel; URU: Urupema; LAG: Lages; SJC: São José do Cerrito; d: diameter at breast height (cm); hd: degree of slenderness; cr: crown ratio (%); cd: crown diameter (m); RF (%): relative frequency of the interval; Prob. > A^2: Probability of the Anderson-Darling statistic associated with the model.

Mathematically, the results indicate that 62.5% of the distribution of the tree's dendro/morphometric variables is described by a probability distribution function (pdf-Weibull), showing that changes in the forest dynamics occur slowly. Dynamics information (stability, productivity, structure, and diversity) are important indicators to manage forest resources (Pretzsch, 2019).

Such a pattern of slow changes in shape and dimension are validated by the probabilities of adjustment ($p > 0.01$) and the Anderson-Darling statistics. Changing the pdf function for the variables indicates that the conditions of the trees and the forest structure are different, since they reflect disturbances that took place in the past (anthropic or natural). They signal possible consequences for the current and future structure of the forest, contributing to forest management planning, if interventions are authorized by federal or state legislation.

Differences in size and shape for some variables are associated with lower/higher site density, growth space between trees, past interventions, competition, and position of the tree in the forest stratum, confirming hypothesis (1) of non-intervention in forest structure. Figures 2, 3, 4 and 5 present the similarities regarding the form of distribution of dendro/morphometric variables and structural stability, showing the non-differentiation in the development stage of natural auracaria forest stands.

Such results are detrimental to the forest ecosystem, since, according to Zeller and Pretzsch (2019), forests currently must produce a quantity of wood and fulfill several ecosystem functions in the same forest stand, simultaneously. The structure and distribution pattern of the variables that characterize it have, therefore, an influence on the diversity and productivity of the forest stand (Bourdier et al., 2016; Danescu et al., 2016; Jacob et al., 2010; Liang et al., 2016; Morin et al., 2011; Paquette & Messier, 2011; Pretzsch, 2013; Soares et al., 2016).

Variable d (Figure 2) showed concentration of trees at the 30 to 60 cm classes, resulting from the last intervention in the forest (in the 90's), favoring space and availability of resources for growth. Trees that currently occupy the strata of codominants and dominants in the forest structure. Diametric distribution indicates competition, reduction in ingrowth rate; also, lower natural regeneration, incidence of light, temperature below the canopy, floristic diversity, stability and complexity and impairment of a future structure.

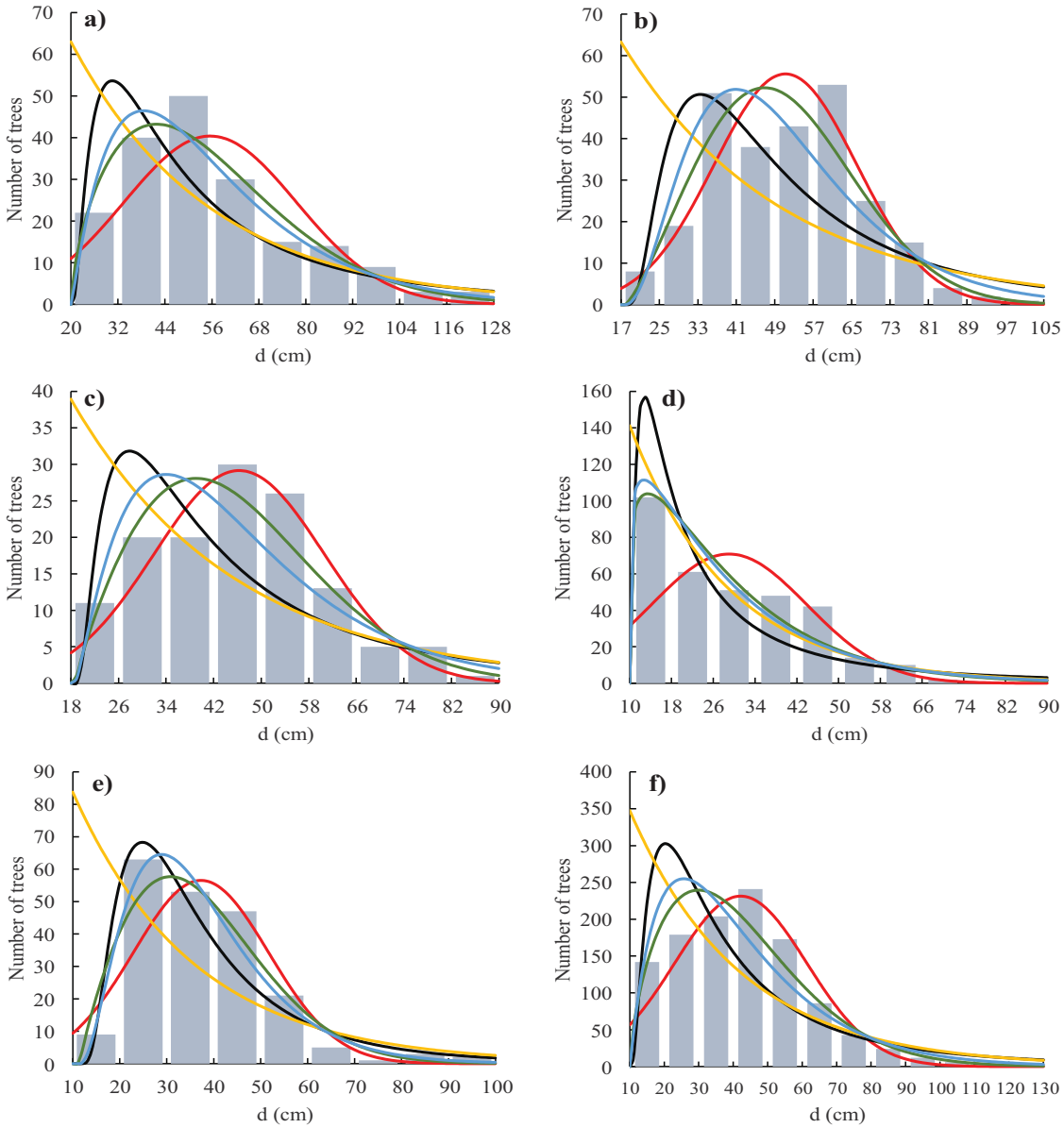

Figure 2. *Araucaria angustifolia* diametric distribution estimated by probability distribution function (pdf) for each site, plotted on their respective frequency histogram: a) São Joaquim (SJQ), b) Panel (PNL), c) Urupema (URU), d) Lages (LAG), e) São José do Cerrito (SJC), and f) All sites. PDF adjusted according to the color lines: red [Normal]; black [Log-normal]; yellow [Exponential]; green [Weibull]; and blue [Gamma]

The results show that there are similarities for the degree of slenderness with a concentration of values not exceeding 55 (Figure 3), meaning that the trees have already established themselves in the forest structure, with less growth in height than in diameter. This corroborates the hypothesis of stability in the vertical structure of the forest and araucaria dominance in the upper canopy.

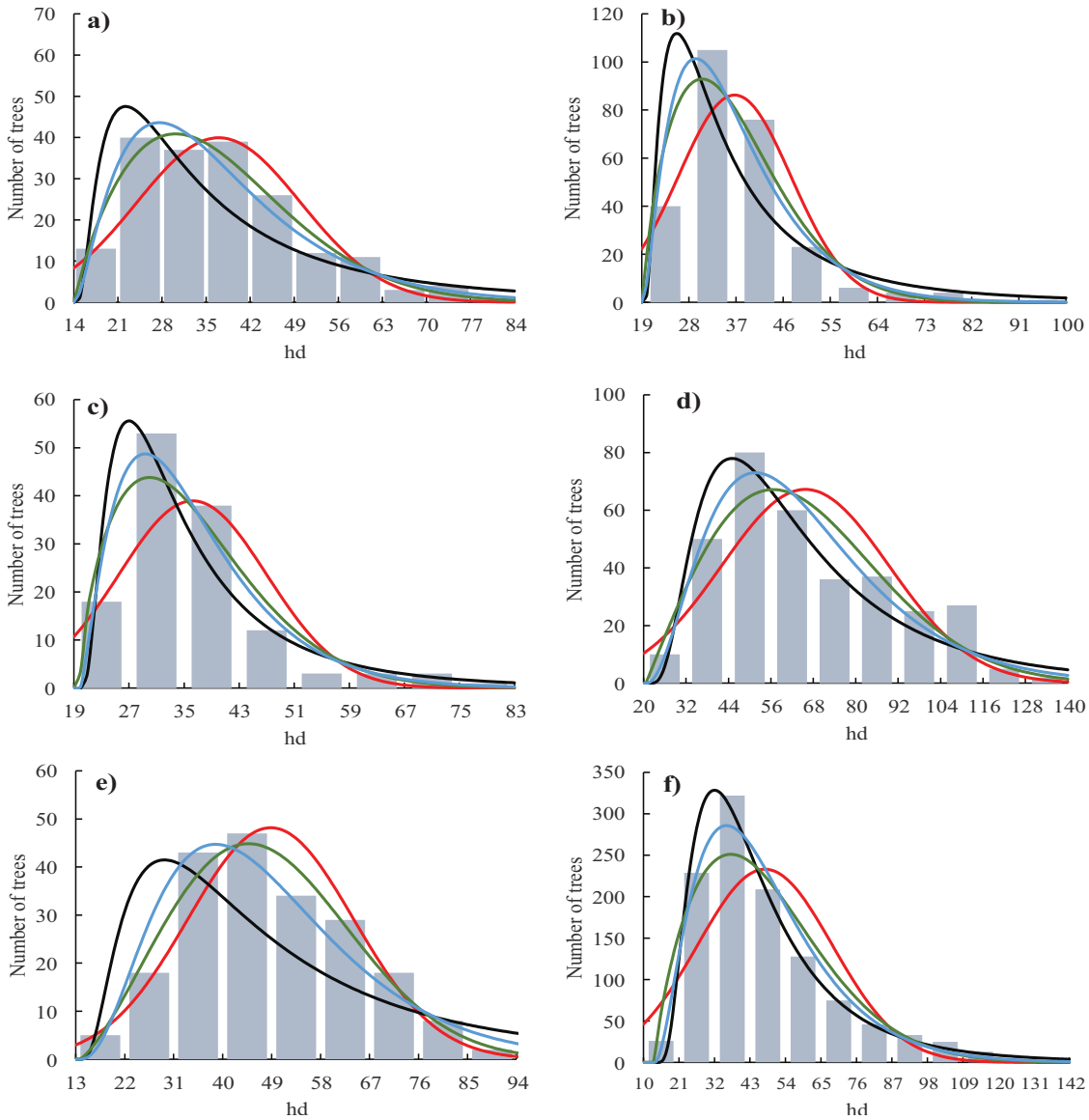

Figure 3. Distribution for the degree of slenderness of *Araucaria angustifolia* estimated by the probability density functions (pdf) tested for each site, plotted on their respective frequency histogram, a) São Joaquim (SJQ), b) Panel (PNL), c) Urupema (URU), d) Lages (LAG), e) São José do Cerrito (SJC), and f) All sites. PDF adjusted according to the color lines: red [Normal]; black [Lognormal]; green [Weibull]; and blue [Gamma]

The distribution of variable cr (Figure 4) also shows a constant pattern, that is, a lack of diversity in the dimension/shape values, which are constant at 35%. This is because cr is a characteristic value of adult stage species, or when the species are found in the dominated stratum, self-draining and stunting its growth. Such aspect, however, has not been proven, since our results show a concentration of trees in larger diameter classes (30-60).

These findings show the relationship with total forest productivity, that is, less cr, less photosynthetic capacity, less carbon absorption, less production. Number of trees with values greater than 45% cr indicate shorter crown length and large width, small formal crown, similar trees and dominant classes. In short, the sites present an increasing lower average rate of diameter (Hess et al., 2018b).

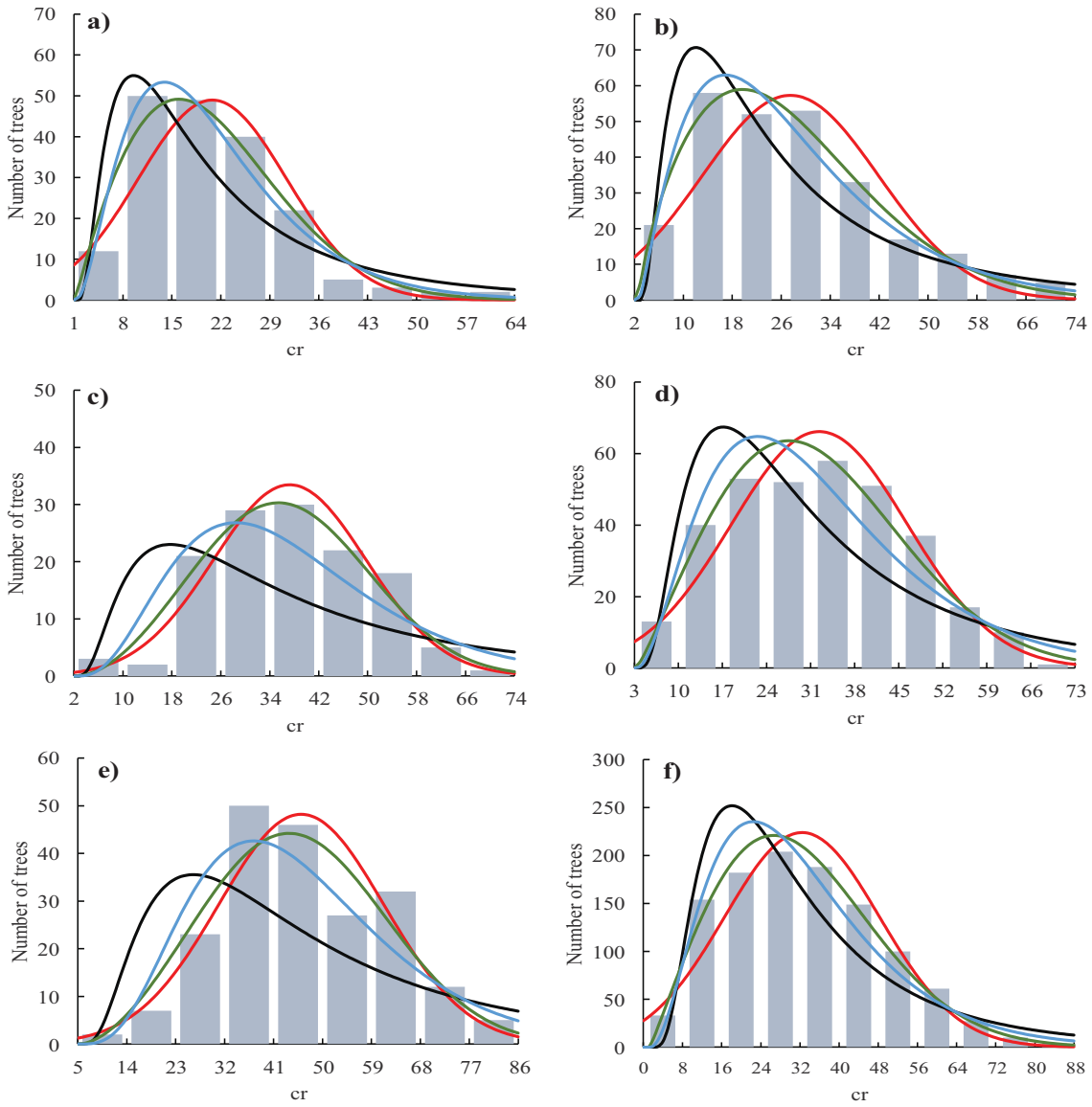

Figure 4. Distribution of the *Araucaria angustifolia* crown ratio estimated by probability density functions (pdf) tested for each site, plotted on their respective frequency histogram: a) São Joaquim (SJQ), b) Panel (PNL), c) Urupema (URU), d) Lages (LAG), e) São José do Cerrito (SJC), and f) All sites. PDF adjusted according to the color lines: red [Normal]; black [Log-normal]; green [Weibull]; and blue [Gamma]

Variable cd presents a distribution that behaves similarly. Since the higher the cd the better the horizontal canopy development and the lower density, the lower value of this variable indicate that trees have less lateral space, greater density and competition. The values for this variable also depend on the position the tree occupies in the canopy, their density and growth rate.

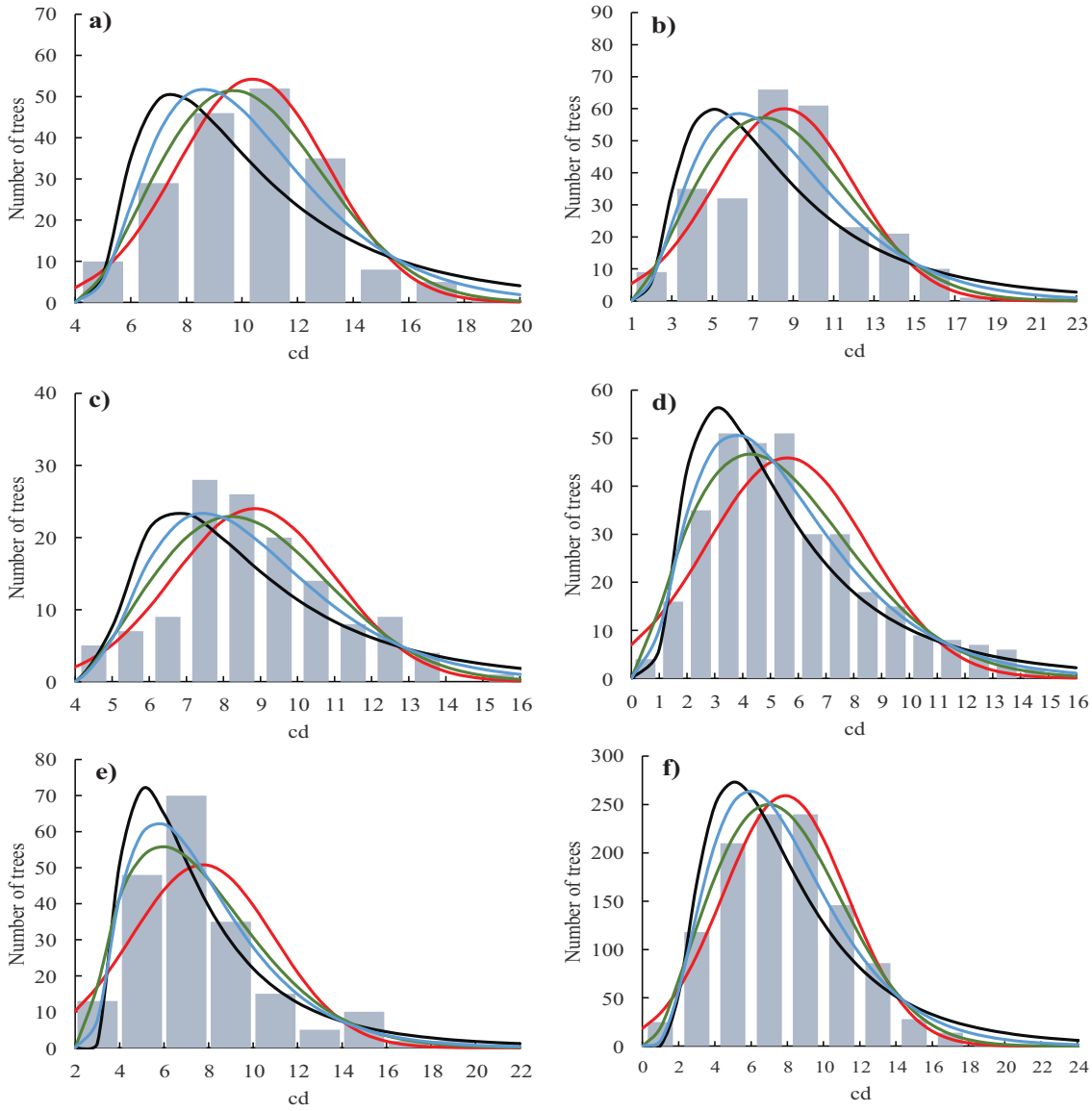

Figure 5. Distribution for *Araucaria angustifolia* crown diameter estimated by probability density functions (pdf) tested for each site, plotted on their respective frequency histogram: a) São Joaquim (SJQ), b) Panel (PNL), c) Urupema (URU), d) Lages (LAG), e) São José do Cerrito (SJC), and f) All sites. PDF adjusted according to the color lines: red [Normal]; black [Log-normal]; green [Weibull]; and blue [Gamma]

4. Discussion

Morphometric analysis allows us to evaluate the dynamics of changes in tree growth and development and ecological processes in forest communities (Mews et al., 2011). Studies on these dynamics are thus possible using the form of distribution of variables such as tree shape and size (dendro/morphometric). This knowledge should be used to aid the conservation and management of forest stands, especially mixed forests.

The similarities, or lack of significant changes, found by this study regarding the form of distribution of the dendro/morphometric variables results from the current and past conditions of tree development, indicating a compromise for a future structure.

Results show a stabilization pattern in the vertical and horizontal structure, with dynamic changes occurring slowly and gradually, or a negative instability. While this stabilization of the dynamics may indicate a lack of planned management, what is certain are its consequences for species conservation, and the stability, diversity, and productivity of forest resources. It is, thus, as harmful to the forest as the irrational use of resources.

The question remains whether to direct (manage) the dynamic processes or allow the forest to regulate itself. In the meantime, however, serious consequences due to competition and other processes may become irreversible. Results show that the largest number of trees is concentrated in the 30-60 cm d classes, structural consequences, and contribute in imbalances in mortality and recruitment rates contribute to the net reduction of total community biomass (Mews et al., 2011).

Another consequence is reflected in the rate of periodic diameter increase, as losses are more abrupt in smaller diameter trees than in those of larger size, due to competitive ability (Hess et al., 2018a). But the initial phase and growth acceleration when under continuous loss influence the tree's complete development. Its reflection on the structure will be greater in the future than the loss in already established trees in the canopy.

In part, our findings contradict the theory that in preserved forests, mortality is balanced by recruitment (Mews et al., 2011). Imbalance, periods of stability or instability in dynamics are rhythmic cycles in undisturbed forests (Sheil et al., 2000). Again, the paradigm reflects the dichotomy of using or not using forest resources. For forest science, forest management is the tool that reduces risks and uncertainties, does not disregard inputs, and favors full social and economic growth.

Considering the lack of intervention in the forest, the trees concentrated in d classes, reaching their maximum morphometric value and occupying the middle and upper strata, which inhibit sunlight entry and increase competition for resources such as space, light, nutrients and water. This competition affects young trees more intensively (Braga & Rezende, 2007), being detrimental to the formation of a future structure (old growth trees).

The lack of differentiation in size and shape constitute a serious issue, since phenotypic plasticity, phenology and canopy architecture are important in evolutionary ecology and in understanding plants, community, forest stand structure and production functioning (Barthélémy & Caraglio, 2007). According to Barthélémy and Caraglio (2007), a plant's architecture depends on the nature and relative arrangement of each of its parts; it is, at any given moment, the expression of a balance between endogenous growth processes and exogenous restrictions exerted by the environment. In forest science, this must be driven and managed by silviculture and forest management over time and space, providing goods and services to society and the ecosystem.

Using information from dendro/morphometric criteria and indices and considering the plant as a whole, these analyses and modeling are essential to understand forest dynamics, development, and management. Morphometric indices with reduced and stabilized values inform that forest dynamics are at a slow pace, which may compromise future structure and diversity, species conservation, and hinder the application of management regimes, especially traditional and known ones. These considerations are evident when analyzing the results of the distribution pattern for the dendro/morphometric variables.

Results show that the lack of structural heterogeneity can negatively affect the functioning of the forest ecosystem, thus confirming the hypothesis of a sustained silvicultural intervention. They indicate the need to open the canopy, remove trees in the classes with higher concentration, thus allowing for sunlight to enter and for regeneration within the species. This contribution is necessary and efficiently assists the processes of species conservation, forest structure, growth and ecosystem resources.

Failure to intervene in dynamic processes evokes detrimental effects regarding forest processes and structure. Removing the canopy through thinning can substantially increase light variability in the understory. Studies suggest that increasing spatial heterogeneity with thinning of different intensities could help restore biodiversity in temperate coniferous forests in northwestern US (Carey 2003; Curtis et al., 1998).

A study by Tsai et al. (2018) showed that thinning creates a link between resource heterogeneity and biodiversity in the understory or legacy effects associated with induced changes in understory regeneration. Studies have shown that, if practiced properly, thinning could facilitate the development of old-growth forest characteristics (O'Hara et al., 2010) and increase biodiversity (Longhi et al., 2018; Pollock & Beechie, 2014).

Interventions are important because size is related to life expectancy, size of living area and other aspects of life history and ecology, the relationship between size and density is an essential link between the individual - and population-level traits of species and the structure and dynamics of ecological communities. In addition, because body size is one of the primary determinants of metabolism and, therefore, resource use, the relationship between size and abundance also reveals how resources are partitioned in ecological systems (White et al., 2012).

In the same way, components of wood quality result from the tree's phenotype, which is determined by both the genotype and the environmental conditions, *i.e.*, the species-specific morphological plasticity and spatial arrangement within the stand (Assmann, 1970). Wide spacing and crown release by heavy thinning can increase

light supply and foster crown width and length. Suppressed trees in the understorey, in contrast, may react to the light limitation by lateral rather than vertical crown extension.

The importance of forest management is in the recognition of identifying in which period of the dynamic which variable is an indicator of the need for intervention. And that needs to be modified in terms of reference value, need for change, in relation to lower or higher density. In short, interventions are also cyclical dynamics, with each variable, over a given period, having an importance in changes in the development of species in the forest.

Examples, studies where density reduction did not affect slenderness or even caused it to increase are exceptions. The crown ratio and crown projection ratio in all reviewed studies increased when stand density was reduced (Pretzsch & Rais, 2016). Indicating that the degree of slenderness has greater significance in the establishment of the tree, mainly in the early and juvenile stages, as crown variables in secondary growth, increase in size and production. What cannot happen is stabilization, concentration in values or dimension, or non-significant changes in time to the response and for the predictor variables in forest management models.

The concentration of values and trees in diameter classes is thus an indication that the MOF currently has a homogeneous structure. Confirming that not managing the stands for a long period of time we can have forests with regular structure having a dominant species. While uneven-aged mixed stands contain a broader spatial variety and more diverse and irregular structures. Uneven-aged pure stands may lie somewhere in between regarding structural heterogeneity.

In mixed forests the variations in the resource supply and of the variation in the morphological shapes, consequently, in the values of the dendro/morphometric variables may be broader. The density of regeneration in the sites is also shown to be lower as a lack of intervention in forest stands, as evidenced also by study of Cavallin and Vasseur (2008).

5. Conclusions

The analysis of the results allows us to conclude that interventions are necessary aiming at the questions of structure, regeneration, conservation, use of resources and changes in dynamics, not in a slow way, but accelerating processes. This is due to the fact that the araucaria has a lifetime that can last more than 200 years, actions of nature, such as opening gaps, with falls from old trees also take time.

Surveys with measurement of dendro/morphometric variables have been carried out by our forest management and growth laboratory for a decade. This helps to conclude that there are no significant changes in the dynamics of the forest with stabilization in its structure for more than 15 years. In a period of 4 years at the LAG site, there was a reduction in the number of trees in the class center by 12.5 cm in diameter (Silveira et al., 2018).

The analyzes of dendro/morphometric variables were carried out in terms of individual trees, indicating that even though the sampling processes at the sites were different, it did not influence the results in terms of reference values, concentration of values, dynamic analysis in terms of structure and yield. The results conclude that the lack of interventions (control thinning) in recent decades has caused harmful consequences in the life cycle of trees, competition, availability of resources, decrease in growth rate, increment and shape, in ecosystem diversity, stability, structure, productivity and conservation of the species.

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First Record of *Sericomyrmex mayri* for Paraguay and Increasing the Range of Distribution of 17 Ant Species in the Central Department

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Abstract

Ants have been studied in Paraguay, South America, over the last two centuries, nevertheless new species can still be discovered with simple sample surveys. Most species collected in the country belong to one or few locations, therefore knowledge about species distribution is limited. A total of 2,040 ants have been collected, belonging to 7 subfamilies and representing 44 species. All of these species, except *Sericomyrmex mayri*, were documented for Paraguay and 17 species were first documented in the Central Department. Those 17 species are: *Camponotus sanctaefidei*, *Crematogaster acuta*, *C. arata*, *Cyphomyrmex laevigatus*, *C. lectus*, *C. minutus*, *Forelius pusillus*, *Linepithema neotropicum*, *L. pulex*, *Mycetomoellerius fiebrigi*, *Nylanderia docilis*, *Pheidole cyrtostela*, *Pogonomyrmex tenuipubens*, *Solenopsis megergates*, *S.richteri*, *Strumigenys hindenburgi*, and *Wasmannia lutzi*. These species belong to 12 genera and 3 subfamilies. The new recorded species is described and illustrated with photographs of the collected specimens as well as a short description of taxonomy, ecology, and distribution. A list of the new species to the Central Department is also provided. The aim of this study is to increase the knowledge of ant species in Paraguay and their distribution.

Keywords: ants, distribution, taxonomy

1. Introduction

Paraguay, South America, is divided into two broad regions by the Paraguay River: The Occidental Region and the Oriental Region. The Central Department is located in the middle of Paraguay with a range of ecosystems, from natural grasslands, gallery forests, flood plains along the Paraguay River to wetlands around the Ypacarai Lake (Avila et al., 2018). It is also the most populated place of the country, as the mayor cities are located within its limits. This means there is considerable pressure on the natural resources as demand for housing, water, energy and waste disposal is increasing. Birds and mammals are among the most studied components of the fauna, but arthropods, especially ants, have been less studied. Worldwide, 12,571 ant species are known to science, belonging to 22 subfamilies (Rabeling et al., 2008), with 3,100 ant species are cited in the Neotropics (Fernandez, 2003). Roughly a sixth of the Neotropic ants live in Paraguay with 541 described species (Wild, 2007a). Using the Chaos-2 statistical estimations, species richness ranges between 698±35 species. Knowledge about ants still remains scattered and incomplete and Paraguay's ant fauna is among the most poorly studied in the Neotropics (Wild, 2007a). Most recent works done by Delsinne et al. (2008, 2010, 2012) have recorded new species of ground dwelling ants for Paraguay. The aim of this study was to describe species of ants in the Central Department of Paraguay.

2. Methods

Field work was carried out between March 2019 and April 2019 in the locality of Cocue Guazú, Areguá, in the Central Department of Paraguay, located at 25°20´48.10´´ South and 57°22´15.84´´East (Figure 1) at an elevation of 172 m.a.s.l. and belonging to the Ecoregion Litoral Central (Avila et al., 2018).

Figure 1. Location of the sample area

Three sample plots on a 50 meter transect were established and every 5 meters a pitfall was installed. The pitfall collection method was chosen as it is the most efficient trap for ground dwelling arthropods (Wiezik et al., 2015; Sheikh et al., 2018). Pitfalls were 7.5 cm in diameter on the top and 12 cm in height. They were filled with a 50% diluted alcohol solution. Traps were collected and reinstalled once a week. The contents were then stored in 80% diluted alcohol in labeled containers. After 4 weeks of collection the resulting 120 containers were filtered and only ant specimens were retained. The ant specimens were identified to genus using morphological keys from Bolton (1994, 1995, 2019) and Baccaro et al. (2015). To identify to species, a catalogue was created based on the species list published for Paraguay by Wild (2007a), and the taxonomic keyy by Fernandez et al. (2003). The collected ant specimens were stored in the Collection of the Facultad de Ciencias Exactas y Naturales (FACEN) of the Universidad Nacional de Asunción (UNA). Measurements and pictures were taken with a BOECO BSZ-450 Stereoscope and a B-Cam 14 Camera. Parameters measured were Total Length (TL), Head Width (HW), Scape Length (SL), Weber's Length (WL), Petiole Length (PL), Hind Femur Length (HFL), Cephalic Index (CI) = (HW/HL) × 100 and Scape Index (SI) = (SL/HW) × 100. A total of 9 queens and workers of *S.mayri* were measured and their average values calculated.

3. Results and Discussion

The genus *Sericomyrmex* belongs to the higher Attini group which are fungus farming ants such as *Atta* and *Acromyrmex* (Ješovnik & Schultz, 2017) which have an important role in nutrient cycling in the ecosystem. Higher Attini means that the ants are polymorphic, presenting certain degree of variation among individuals of the same colony. They provide their fungal gardens with fresh vegetal material, therefore ant species belonging to this group are of special interest to agricultural and forest plantations. Even though the genus *Sericomyrmex* was first described by Mayr (1865), it was not until 2017 that a full set of taxonomic keys and a clear species description was available (Ješovnik & Schultz, 2017). The previous authors described 11 species and 3 subspecies from Mexico to southern Brazil. *Sericomyrmex* lives in a variety of habitats, such as deserts, savannah, forests, and urban areas (Mehdiabadi & Schultz, 2010).

In Paraguay, the only documentation of this genus was *Sericomyrmex scrobifer* collected in the Department of Canindeyú by Alexander Wild in 2002 (Ješovnik & Schultz, 2017).

S. mayri has been reported in northern Bolivia, Colombia, Ecuador, Peru, the Guianas, and throughout Brazil (Figure 2, Ješovnik & Schultz, 2017). The specimens collected in Paraguay therefore represent the southernmost location of this species.

Figure 2. Known distribution of *S.mayri* (Ješovnik & Schultz, 2017)

As only one species of this genus has been documented for Paraguay, this study may prompt the discovery of larger ant species.

The other 17 species first recorded for the Central Department belong to 12 genera and 3 subfamilies. Despite this study's short period of sampling, numerous species were recorded for the first time in this part of Paraguay. Table 1 shows where ant species have been recorded previously and by whom. Recommended for future Paraguay ant study is more samplings in different ecoregions of the country, and molecular biology analysis. Additionally, future research should include country-wide ant species distribution, ecology, and the impact of land use and climate change on species composition.

3.1 Taxonomy

(1) *Sericomyrmex mayri* (Forel, 1912)

Species description from Ješovnik and Schultz (2017): Large species; head broad; frontal lobe narrow, directed anterad; mandible usually striate; frontal carina often reduced, incomplete; eye flat to mildly convex; posterior cephalic margin shallow, abruptly to gradually impressed; posterior cephalic corner usually angled; mesosomal tubercles low and obtuse, first gastral tergite with lateral carina well developed, dorsal carinae absent or faint. Body covered with hair, which gives a silky appearance to the ant. This is where the name *Sericomyrmex* is derived from as Latin "sericeus" means "silky".

(2) Worker Description

Measurements in mm, range: TL 6.25-7.88 (7.11), HW 1.81-2.56 (2.12), HL 1.63-2.32 (1.93), SL 1.39-1.95 (1.60), WL 2.26-3.24 (2.76), PL 0.54-0.76 (0.64), HFL 2.07-3.03 (2.51), CI 104-115 (110), SI 70-81 (75) [N = 50]. *Head*: Wider than long (CI = 110±5). Mandibles with 7-8 teeth and striated laterally. Eye slightly convex, medium-sized. Antennal scape short (SI = 75±5). *Pilosity*: Body, head and legs covered with dense flexuous pubescence. Hairs curved, darker at the base, white to yellow. *Mesosoma*: Propodeal carinae flat, mesosomal tubercles not pronounced. *Metasoma*: Petiole and post petiole both with a longitudinal serrated carinae (Figure 3).

3.2 Ecology

S. mayri is difficult to observe in their natural habitat, as they move slowly and their color camouflages them. When these ants are disturbed, they stop moving unlike running to their nests like other ground-dwelling ant species. The plot where all the *S. mayri* specimens were collected had a continuous forest cover, constituted mainly

of Kupa'i (*Copaifera langsdorfii*), Kurupa'y kurú (*Anadenanthera colubrina*), Tataré (*Chloroleucon tortum*), Yvyra ro (*Pterogyne nitens*) and a few specimens of Mbokaya palm trees (*Acrocomia aculeata*).

Figure 3. *Sericomyrmex mayri* worker

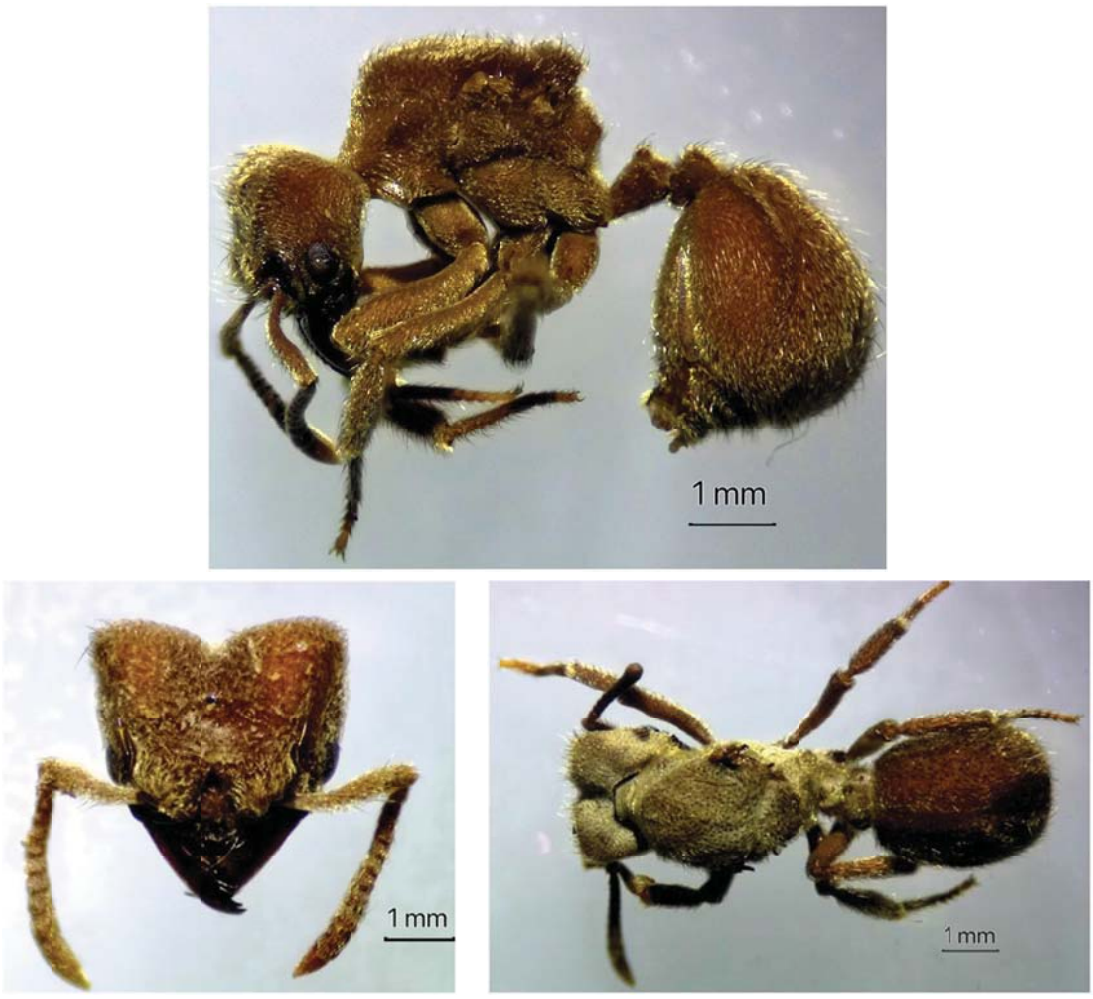

Figure 4 *Sericomyrmex mayri* queen

(1) *S. mayri* Queen Description

Measurements in mm, range: TL 8.05-9.13 (8.89) HW 3.05-3.17 (3.1) HL 2.88-2.98 (2.93) SL 2.25-2.69 (2.53) WL 3.85-4.02 (3.94) PL 0.91-0.96 (0.94) HFL 3.02-3.22 (3.12) CI 102-108 (106) SI 74-84 (82) [N = 8] (Figure 4).

Table 1. New species recorded for the Central Department of Paraguay

	Subfamily	Species	Distribution in Paraguay	References
1	Dolichoderinae	*Forelius pusillus* (Santschi)	CA	Wild, 2007a
2		*Linepithema neotropicum* (Wild)	CA, AP	Wild, 2007b
3		*Linepithema pulex* (Wild)	CA	Wild, 2007b
4	Formicinae	*Camponotus sanctaefidei* (Dalla Torre)	CG, CA, ÑE, SP	Wild, 2007a
5		*Nylanderia docilis* (Forel, Brandão)	CA	Wild, 2007a
6	Myrmicinae	*Crematogaster acuta* (Fabricius)	CA	Wild, 2007a
7		*Crematogaster arata* (Emery)	CA, GU, MI	Wild, 2007a
8		Cyphomyrmex laevigatus (Weber)	CA	Wild, 2007a
9		*Cyphomyrmex lectus* (Forel)	CO	Wild, 2007a
10		*Cyphomyrmex minutus* (Mayr)	CA, MI	Wild, 2007a
11		*Mycetomoellerius fiebrigi* (Santschi)	1916-Santschi no location mentioned	Santschi, 1916
12		*Pheidole cyrtostela* (Wilson)	AM	Wilson, 2003
13		*Pogonomyrmex tenuipubens* (Santschi)	CG	Fowler, 1981; Taber, 1998
14		*Solenopsis megergates* (Trager)	BO	Trager, 1991
15		*Solenopsis richteri* (Forel)	ÑE	Fowler, 1981
16		*Strumigenys hindenburgi* (Forel)	IT	Fowler, 1981
17		*Wasmannia lutzi* (Forel)	IT	Forel, 1908

Note. Names of the Paraguayan Departments are abbreviated as follows: AP: Alto Paraguay; AR: Alto Paraná; AM: Amambay; BO: Boquerón; CG: Caaguazú; CZ: Caazapá; CA: Canindeyú; CE: Central; CO: Concepción; CR: Cordillera; GU: Guairá; IT: Itapúa; MI: Misiones; ÑE: Ñeembucú; PR: Paraguarí; PH: Presidente Hayes; SP: San Pedro.

4. Conclusion

A total of 2,040 ants were collected in this study, belonging to 7 subfamilies and representing 44 species. All of these species, except *Sericomyrmex mayri* have previously been documented in Paraguay, though this study is the first to document all 17 species in the Central Department of Paraguay. These results suggest that ant diversity in Paraguay is largely underestimated. Future studies concerning this important arthropod group may uncover additional information regarding ranges of known species and locating heretofore unknown species nationally.

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Prevalence and Incidence of Cassava (*Manihot esculenta*) Brown Leaf Spot Disease Caused by *Cercospora heningsii* in Macuata Province, Vanua Levu, Fiji

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Abstract

Cassava (*Manihot esculenta Crantz*) is a crop of many values in the tropical and subtropical regions of the globe. In Fiji, cassava is cultivated on vast acres of land but, the yield obtained is relatively lower because of many constraints, including the prevalence of diseases caused by the different pathogens. Among various pathogens responsible for a lower yield, the cassava brown leaf spot disease caused by *Cercospora heningsii* is responsible for causing enormous annual losses of cassava in tropical and subtropical regions. Because there is very little information regarding the association of the brown leaf spot disease and cassava in the country, the present study using survey as research instrument endeavors to determine the disease incidence and prevalence of brown leaf spot disease in the cassava fields of three villages (Mani Road, Boca, and Anuve) in the Bulileka area of the Macauta province in Vanua Levu, Fiji. The study found that brown leaf spot disease prevailed (100%) in all three villages. The percentage of disease incidence ranged from 36.4% to 42.9%. The maximum incidence (42.9%) of cassava brown leaf spot disease was found in Anuve village, followed by Mani Road village (38.2%), with the lowest disease incidence recorded for Boca village (36.4%).

Keywords: prevalence, incidence, brown leaf spot, cassava disease, *Cercospora heningsii*, Fiji Islands

1. Introduction

Cassava (*Manihot esculenta* Crantz) is a shrubby perennial plant of the family Euphorbiaceae that typically grows from one to three meters (3-10 feet) in height (Thresh et al., 1998). Among the 28 known species of the Euphorbiaceae family, cassava is the only edible crop, and its tuberous roots are a good source of carbohydrates (Katz & Weaver, 2003). Cassava is a key staple food in several countries (African, South American, Asian, and the Pacific) and has the highest production potential calories per hectare per day among tropical crops (Alicai et al., 2007). In Fiji, cassava is grown by small-scale farmers for subsistence use and its cultivation often also constitutes an essential source of income in rural and marginal areas.

However, cassava growth and yield are affected by biotic constraints (Hahn et al., 1989), among which pathogenic diseases are of critical importance. In Fiji, a primary fungal disease that affects cassava production is the brown leaf spot disease caused by *Cercospora heningsii* (Tsatsia & Jackson, 2010). The symptoms of this disease appear as small brown spots with dark borders on the upper surfaces of the leaves (Msikita et al., 2000). On the underside of the leaf, the disease spot displays greyish color with less distinct borders, with minor veins crossing the disease spots and appearing as black necrotic lines. The center of the disease spot is dry, looks cracked, and appears as if it will suddenly fall off (Tsatsia & Jackson, 2010).

The cassava leaves are the core source of assimilation for dry matter production. An increase in brown leaf spot disease reduces the photosynthetic area and capabilities of the plant (Hahn & Hozyo, 1984). A severe brown leaf spot infection results in total defoliation and hence failure (Alabi & Waliyar, 2004). Similarly, many of the leaf spot-causing organisms can kill the host partially or fully, not only by direct destruction of the tissues but also by systemic dispersal of toxic substances far beyond the original areas of infection (Bilgrami & Dube, 1976).

The amount of disease is measured as the proportion of the crop population (counted as individual plants or branches or leaves) that is infected (disease incidence) or the percentage of the area of the plant that is affected (disease severity). Several studies relating to the cassava brown leaf spot disease exist in many tropical and subtropical countries (Pei et al., 2014; Powbunthorn et al., 2012; Banito et al., 2007). However, there are few or no studies conducted to ascertain the incidence and prevalence of the disease on cassava plants in Fiji. Therefore, the present study was conducted to determine the incidence and prevalence of the disease in three villages at the Macuata Province of Fiji.

2. Methods and Materials

2.1 Study Area

A survey of cassava fields located in three selected localities of the vegetable-growing villages in the Macuata Province of Labasa was conducted in the year 2014. The sites were selected based on the following factors. First, the villages could be easily accessed by the researchers. And second, they were selected based on experiences and observations shared by local farmers that the disease was becoming widespread in their cassava fields.

2.2 Map of Study Area

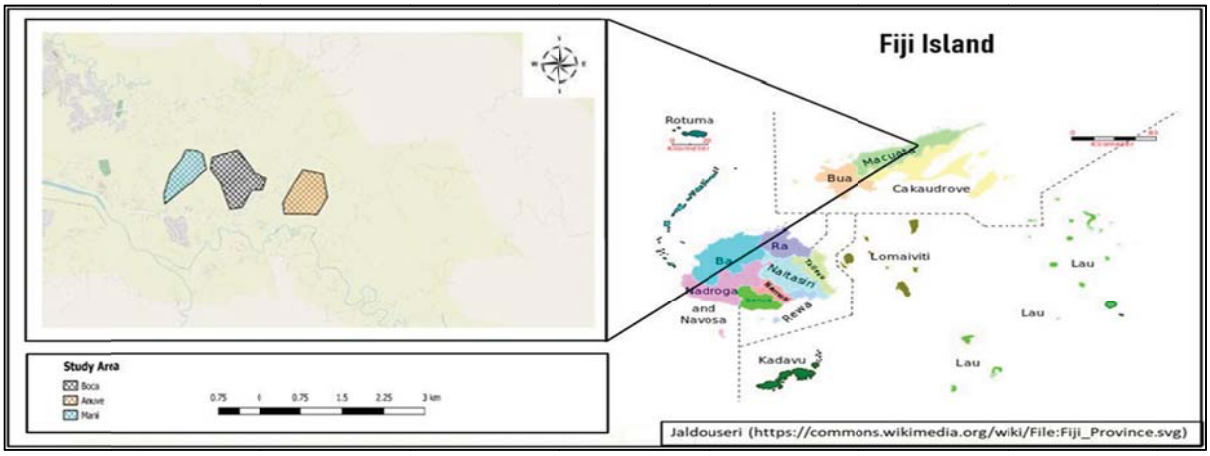

Figure 1. Map of study area

Note. Three villages (area) selected for this study are indicated using closed borders.

2.3 Data Collection

To determine the prevalence and incidence of brown leaf spot disease, three cassava fields, each from Mani Road, Boca, and Anuve, were selected using a simple random sampling (SRS) approach (Banito et al., 2007). After randomly selecting the cassava fields, the study adopted the data collection approach (Z transect method) used by Hussain et al. (2012). From each cassava field, 20 cassava plants were selected to determine the incidence of the disease. To detect the disease, cassava leaves were inspected, and observations made were scrutinized against information such as disease characteristics and symptoms found in the Pacific Pests and Pathogens Fact Sheets by Tsatsia and Jackson (2010).

2.4 Incidence of Disease

Disease incidence is commonly used to refer to the number of a specific part of a plant that is affected by the disease. The specific part of the plant can be its roots, stem, and leaves (Campbell & Neher, 1994). For this study, the incidence of the disease is based on its visibility on leaves. Disease incidences (%) are calculated by taking the number of leaves infected divided by the total number of leaves being observed and multiplied by 100 (Equation 1).

$$\text{Incidence (\%)} = \frac{\text{Total No. of infected leaves}}{\text{Total No. of leaves observed}} \times 100 \quad (1)$$

2.5 Prevalence of Disease

Prevalence of the disease is defined by the incidence of the disease visible on plants in a particular geographic area (Nutter et al., 1991; Paparu et al., 2018). The disease prevalence was calculated by taking the number of

fields displaying incidence of the disease divided by the total number of fields assessed and multiplied by 100 (Mounde et al., 2009).

$$\text{Prevalence (\%)} = \frac{\text{Number of disease fields}}{\text{Number of fields assessed}} \times 100 \quad (2)$$

3. Results and Discussion

3.1 Detection and Inspection

After conducting the inspections, we confirmed that the brown leaf spot disease was present in the cassava fields of the three villages. The disease was mostly found on mature and old plants but rarely on young plants (Figure 2). The characteristics of the disease in the fields was matched against the descriptions and facts outlined by Tsatsia and Jackson (2010) in the Pacific Pests and Pathogens Fact Sheets to further confirm its presence.

Figure 2. Cassava leaves with visible brown spots caused by *Cercospora heningsii*

3.2 Incidence of the Disease

Disease incidence was calculated using Equation 1 for the three villages: Mani Road, Boca, and Anuve. The calculations showed that Anuve village had the highest incidence (42.9%) of cassava brown leaf spot disease, followed by Mani Road village (38.2%). The lowest incidence of the disease was found at Boca village (36.4%) (Table 1).

Table 1. Summary of cassava brown leaf spot disease incidence (%) in three villages of Bulileka

Village	Total No. of cassava plants surveyed	Total No. of Leaves inspected	Total No. of infected leaves inspected	% disease incidence
Anuve	20	35	15	42.9 %
Mani road	20	34	13	38.2 %
Boca	20	33	12	36.4 %
Average				**39.2 %**

Generally, lower severity and incidence of cassava brown leaf spot disease may be due to climatic conditions (Takatsu et al., 1978). Our results overall indicated a high incidence of the disease for the three villages. However, when we compare the three villages, there is minimal deviation. Higher disease incidence may be due to the continuous introduction of infected planting materials brought and utilized by farmers from the infected areas of the farm (Banito et al., 2007). In addition, a high incidence of cassava brown leaf spot disease may also be attributed to the cultivation of a single crop. Hiddink et al. (2005) reported that continuous cropping of one specific crop leads to increased disease incidence, often caused by soil-borne and other plant pathogens. Despite showing an insignificant variation of disease incidence within the three villages, some parameters may have

influenced the disease incidence in these three villages to vary. Banito et al. (2007) reports that environmental conditions such as temperature, humidity, soil type, soil moisture, and cropping patterns are important parameters that influence the incidence of this disease.

Although we have observed higher disease incidence in all three villages, the percentage of disease incidence is below 50%, meaning that more than 50% of the cassava plants are unaffected by the brown leaf spot disease, which explains why the Crop Farmer's Guide for Farmers formulated by the Ministry of Agriculture, Government of Fiji indicated that there is no significant disease that threatens cassava in Fiji (Fiji Government, 2015).

3.3 Prevalence of Cassava Brown Leaf Spot Disease

The prevalence of cassava brown leaf spot disease for the three villages was calculated using Equation 2. Results reveal a 100% disease prevalence for all the three villages (Table 2).

Table 2. Summary of cassava brown leaf spot disease prevalence (%) in three villages of Bulileka

Village	Number of cassava fields	Number of cassava field infected	% disease prevalence
Anuve	3	3	100 %
Mani road	3	3	100 %
Boca	3	3	100 %
Average			**100 %**

High disease prevalence can be attributed to the susceptibility of cassava plants to the disease and the availability of a conducive climate for the disease to proliferate. For example, its prevalence in the study areas can be attributed to weather parameters such as the wind (moderate to high), which all three villages experience every day. In their study, Pelczar et al. (2020) and Tsatsia and Jackson (2010) found that wind was responsible for carrying disease spores across fields and/or from one field to another, and these spores infected cassava plants. In addition, because of tropical climate that Fiji enjoys, the study areas are also exposed to high humidity. Garcia-Guzman et al. (2016) write that humidity also increases the prevalence of the disease in the fields. Therefore, a study as such helps validate the many reports that say that the brown leaf spot disease could be prevalent around the globe where cassava grows (Pei et al., 2014; Powbunthorn et al., 2012; Banito et al., 2007), as we have seen in the case of Fiji for the villages understudy.

3.4 Implication of Study, Limitation, and Further Studies

The impacts of the cassava brown leaf spot disease on cassava yield is widely reported (Elegba et al., 2013; Tsatsia & Jackson, 2010; Otim-Nape et al., 1997; Terry & Hahn, 1980; Terry & Oyekan, 1976). High disease incidence and prevalence and as well improper controls and management by the farmers can lead to yield losses if left unattended. Loss of yield will not only threaten food security but also income security in the villages of Mani Road, Boca, and Anuve, and the same can be said for other villages in Fiji where cassava is cultivated and may also be victims of the disease but are unaware of it. Furthermore, since the current climate of Fiji (Australia Bureau of Meteorology and CSIRO, 2014) is conducive for its proliferation (Tsatsia & Jackson, 2010), we suspect that other parts of Fiji may be facing similar issues.

The limitation of this study is that it does not measure the climatic conditions of the study area for supplementary explanations of the variation in incidence, and the prevalence of the disease at the Mani Road, Boca, and Anuve villages. Also, the number of study sites and the total number of plants selected are too small to determine the overall incidence and prevalence of cassava brown leaf spot disease for the whole of Fiji. However, this can be done by increasing the number of study sites and the selection of reasonable representative sample of plants. Further studies can be undertaken to determine disease severity, impacts on yield, and its management. Also, it will be worthy to evaluate the current and future effects of climate change on disease incidence, severity, prevalence, and its implications on food and income security of local cassava farmers in Fiji.

4. Conclusion

Cassava brown leaf spot disease was prevalent in this study, 100 percent for all three villages, while the disease incidence varied across the three villages. Cassava plants showed extremely severe brown leaf spot symptoms in the cassava fields, raising the fear of high yield losses. Therefore, it is essential to conduct regular inspection and monitoring of cassava brown leaf spot disease to ensure the application of correct disease management strategies, and for successfully increasing crop yields.

Disclaimer

This paper represents the views of the authors and not that of their employers.

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Appendix A

Disease Incidence Calculation

Table A1. Percentage disease incidence of cassava brown leaf spot at Mani road village

Village 1	**Mani road**		
Plant No.	**Number of leaves per plant**	**Number of infected leaves per plant**	**% Disease incidence**
1	38	12	31.6%
2	40	13	32.5%
3	33	12	36.4%
4	35	14	42.9%
5	36	12	36.1%
6	40	18	47.5%
7	42	18	47.6%
8	40	12	30%
9	22	9	40.9%
10	20	7	35%
11	25	10	40%
12	24	9	37.5%
13	28	12	46.4%
14	30	11	40%
15	28	15	53.5%
16	40	14	37.5%
17	40	18	50%
18	41	19	48.8%
19	38	14	42.1%
20	40	11	30%
Total (average)	**34**	**13**	**38.2 %**

Table A2. Percentage disease incidence of cassava brown leaf spot at Boca village

Village 2	**Boca**		
Plant No.	**Total no. of leaves per plant**	**Total no. of leaves infected per plant**	**% Disease incidence**
1	35	13	37.1%
2	30	12	40%
3	31	18	58.1%
4	28	7	25%
5	27	11	40.7%
6	40	17	42.5%
7	28	11	39.3%
8	38	9	23.7%
9	35	8	22.9%
10	31	13	41.9%
11	32	12	37.5%
12	45	17	37.8%
13	44	19	43.2%
14	36	9	25%
15	33	13	39.4%
16	30	12	40%
17	20	7	35%
18	22	7	31.8%
19	30	9	30%
20	37	16	43.2%
Total (Average)	**33**	**12**	**36.4%**

Table A3. Percentage disease incidence of cassava brown leaf spot at Anuve village

Village 3	**Anuve**		
Plant No.	**Total no. of leaves per plant**	**Total no. of leaves infected per plant**	**% Disease incidence**
1	34	16	47.1%
2	28	12	42.9%
3	34	15	44.1%
4	42	23	54.8%
5	38	14	36.8%
6	40	17	42.5%
7	35	15	42.9%
8	26	11	42.3%
9	27	7	25.9%
10	29	7	24.1%
11	38	9	23.6%
12	36	9	25%
13	39	22	56.4%
14	42	20	47.6%
15	41	26	63.4%
16	28	10	35.7%
17	34	15	44.1%
18	34	17	50%
19	35	15	42.9%
20	40	20	50%
Total (Average)	**35**	**15**	**42.9%**

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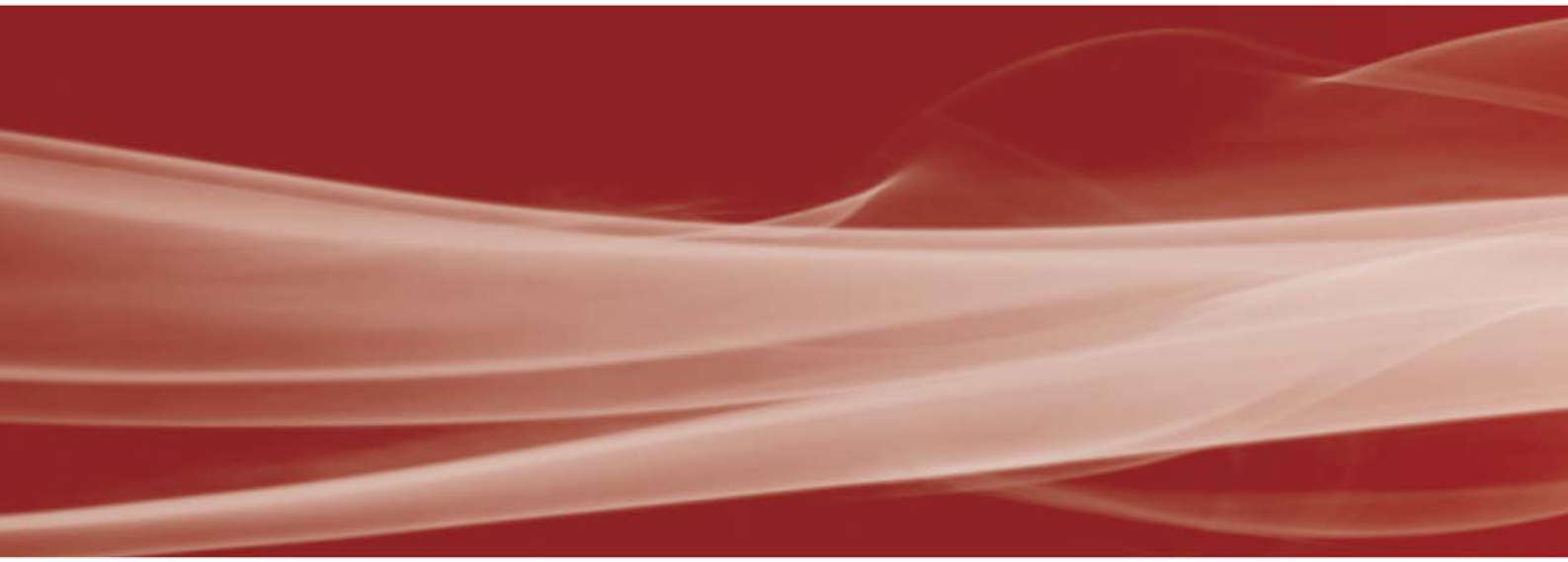

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