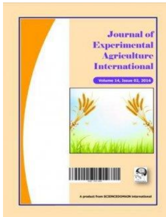

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# In-row Vegetation Cover and Its Effects on 'Tannat' Canopy and Grapes in the *Campanha* Region of Rio Grande do Sul State

**C. S. P. Lamela[1], G. P. Zandona[1], V. B. Costa[2], M. Ferrer[3], M. B. Malgarim[1], P. C. Mello-Farias[1*] and F. G. Herter[1]**

*[1]Faculty of Agronomy Eliseu Maciel, Federal University of Pelotas, Pelotas, RS, Brazil.*
*[2]Federal University of Pampa, Dom Pedrito, RS, Brazil.*
*[3]Faculty of Agronomy, Republic of Uruguay University, Montevideo, Uruguay.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author CSPL conducted field and laboratory work and wrote the first draft of the manuscript as part of his graduate research towards a Ph.D. degree. Author GPZ assisted laboratory work. All other authors contributed equally in data collection, manage the study analyses and field coordination. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** The objective of this study was to evaluate the use of a permanent resident in-row cover crop and its effects on canopy and grapes of 'Tannat' vineyard.
**Study Design:** The experiment was a randomized block with four replications. The treatment factor was permanent resident vegetation cover with two levels, presence of in-row vegetation cover (VC) and vegetation cover absence, with herbicide usage (HB).
**Place and Duration of Study:** Experiment was carried out in a commercial vineyard using a vertical shoot position (VSP) system on Eutrophic Red-Yellow Latosol soil in the city of Bagé, in 2014/2015 and 2015/2016.
**Methodology:** Plant technical, physicochemical and polyphenolic potential parameters related to branch behavior and grape composition were evaluated.

**Corresponding author: E-mail: mello.farias@ufpel.edu.br;*

**Results:** The use of permanent resident in-row vegetation cover slightly decreased its performance during the 2014/2015 crop, but raised quality parameters such as reducing sugars, total and extractable anthocyanins and reduced the potassium content in grapes. During 2015/2016 crop, there were no yield differences between VC and HB, and yet the permanent resident in-row vegetation cover raised the quality parameters in relation to 2014 crop.
**Conclusion:** This technique, where is particularly used in deep soils of the *Campanha* region, promotes sustainability in soil conservation and also can reduce herbicides costs, green pruning, and, mainly, thinning usage when aiming to increase the quality.

*Keywords: Green manures; soil management; vigor control; Vitis vinifera.*

## 1. INTRODUCTION

In-row herbicides usage has been a common and systematic practice in vineyards, but this application is being restricted each day due to the emergence of herbicide-resistant species registered for vineyards. In addition, physiological imbalances by these herbicides imply in environmental consequences such as soil degradation [1,2].

Regarding specifically to glyphosate resistance, in the United States, only three years of continuous glyphosate usage as post-emergence control alternative to resistant cultures, were sufficient to cause the first control failures due to resistance [3].

In Brazil, the resistance to glyphosate, which is the main herbicide used in viticulture, has been reported in several weed species such as *Conyza bonariensis* (hair fleabane) and *Lolium multiflorum* (ryegrass) [4,5].

*Conyza bonariensis* and *Conyza canadensis* are some of the most problematic species in relation to management of perennial crops and specific treatment is required for their control [3,4,6,7].

Besides reducing weeds and improving soil physical properties, cover crops can regulate vineyard growth, decrease vigor, and increase sugar, anthocyanins and polyphenols contents as well as decrease nitrogen and potassium concentration in grapes [8,9,10,11]. Reducing vigor can also have positive effects such as canopy opening, improving sun exposure, reducing shading, and decreasing *Botrytis cinerea* infections [12].

In viticulture, vegetative cover management can control the excessive vigor of the plants and improve the harvest quality, mainly in varieties for wine destiny [13].

In regions where the pluviometric regime is variable, it is difficult to control vigor solely through choosing rootstocks, pruning levels and training systems [13]. The use of permanent resident vegetation in-row as well as inter-row is an alternative for grapes producers, characterized by excessive vigor, and soils with depth and capacity of storage water sufficient not to cause a stress that damages the vineyard. [14,15].

Herbicide usage in the inter-rows, decreased the biopores, macroporosity, total porosity and bulk increase density, indicating topsoil layer compaction. The mechanical mowing of cover crops increased soil aggregate stability in relation to desiccation [16].

Cover crops have been largely used in vineyards soil management as an environmentally sustainable tool for diverse purposes. A major limitation for cover crop use is the additional water consumption. Native grasses adapted to low water availability may be a feasible alternative under drip irrigation [17].

Thus, observing problems and possible paradigm changes regarding soil management in vineyards, the objective was to evaluate permanent resident vegetation cover usage and its effects on canopy and grape of 'Tannat' vineyard in the *Campanha* region of Rio Grande do Sul state.

## 2. MATERIALS AND METHODS

The experiment was conducted during 2014/15 and 2015/16 productive crops, in a commercial field (31º13’55”S, 53º58’53”W, 353 meters above sea level) at Bagé city, RS, Brazil. Climate classification of the region according to Köppen and Geiger [18] is Cfa type, temperate humid with hot summers and chilling hours accumulation (CH) that range from 351 to 400 CH [19].

Local soils belong to the Santa Tecla Unit and is classified as Eutrophic Red-Yellow Latosol soil [20], with clay-sand texture, 18% clay, at 1.2 m depth. The land slope is 1.2%. The soil of the experimental area was analyzed for chemical and physical characteristics after the experiment (Table 1). Permanent resident vegetation in the vineyard is native with predominance of spring-summer species such as *Paspalum dilatatum* Poir., *Paspalum distichum* L., *Paspalum notatum* Flüggé, *Conyza bonariensis* (L.) Cronquist and *Desmodium incanum* DC. There is presence of exotic vegetation (10%), with predominance of *Cynodon dactylon* (L.) Pers. *Trifolium repens* (L.) *Pennisetum clandestinum* Hochst. Ex. Chiov., *Lolium multiflorum* Lam. and *Eragrostis plana* Nees.

Minimum and maximum temperature from local data, rainfall, potential evapotranspiration and water deficit during the experiment were obtained from the Meteorological Station, located in Bagé - RS [21] (Fig. 1). Soil water storage during vegetative crop was also calculated from the Decision Support System in Agriculture data [21] (Fig. 2).

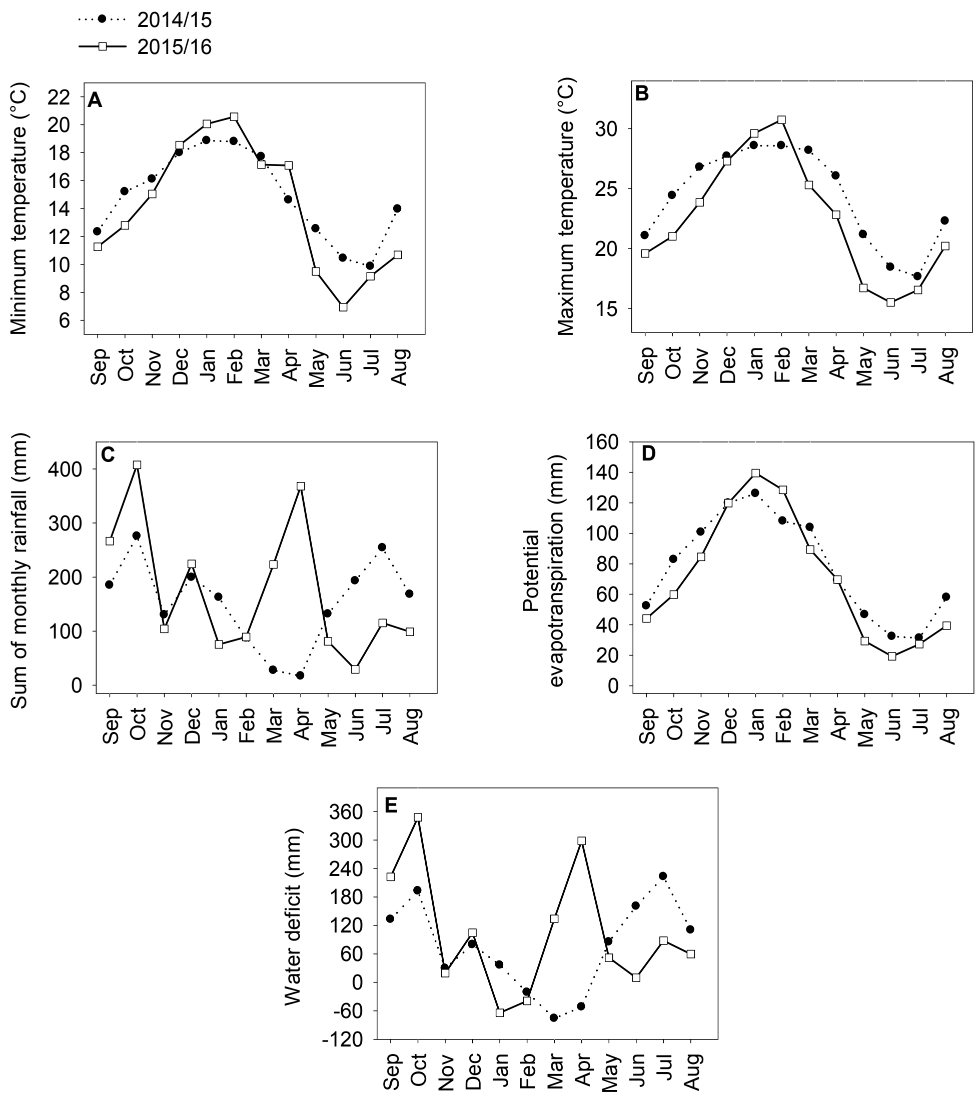


**Fig. 1. Monthly average of minimum (A) and maximum (B) temperatures, monthly averages, sum of monthly rainfall (mm) (C), potential evapotranspiration (mm) (D) and water deficit (mm) (E) between the months of September and August of 2014/15 and 2015/16, obtained from Meteorological Station of Bagé, RS**

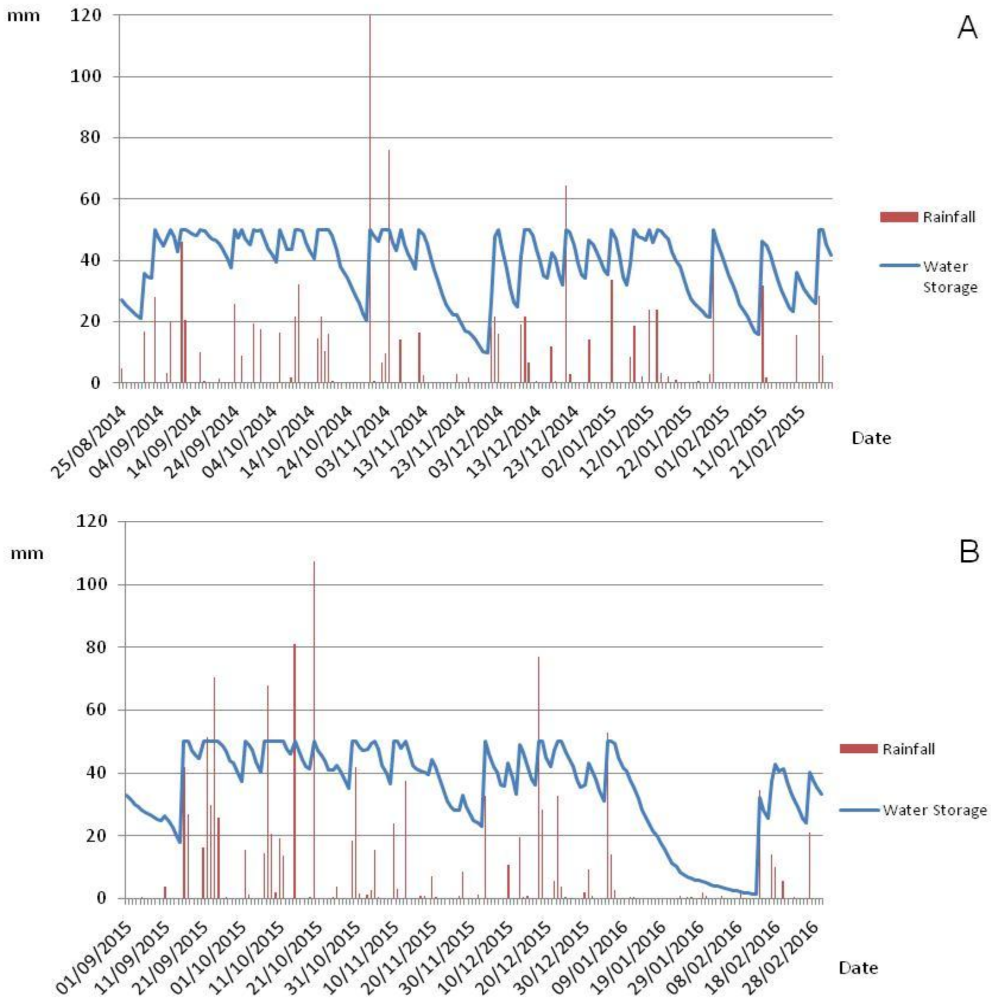


**Fig. 2. Water storage in the soil and rainfall (mm), obtained between September and March 2014/15 (A) and 2015/16 (B), from Meteorological Station of Bagé, RS**

**Table 1. Phenological evaluations according to Eichhorn and Lorenz [20] scale for 'Tannat' grapes during 2014/15 and 2015/16 crops. Bagé-RS**

| | 50% Budding | 50% Flowering | 50% Onset of maturation | Harvest |
|---|---|---|---|---|
| | **5** | **27** | **35** | **38** |
| **2014/15** | 09-Sep | 20-Oct | 13-Jan | 1-Mar |
| **2015/16** | 25-Aug | 12-Nov | 13-Jan | 1-Mar |

The 15-year-old vineyard is placed on North-South orientation, with Tannat cultivar grafted on Paulsen 1103 rootstock, trained in the vertical shoot position (vsp) system, with 1.2 m in-row spacings and 3.3 m between rows. Guyot double was the training system used with two canes of seven buds and two spurs of two shoots. Since 2012, the vegetation has remained spontaneous in the vineyard as well as in-row, maintaining the vegetation length.

Experimental design consisted of a randomized block design, with four replications and one plant being the experimental unit. Treatment factor was permanent resident vegetation cover with two levels, one of the levels being the in-row presence of the vegetation cover (VC), and the other, the vegetation cover absence using herbicide (HB). The VC was defined as plants with permanent resident vegetation cover, which was cutted twice with a brush cutter (Sthil Skim

4300), first in July and the second time during pre-flowering at the 17th stage of the Eichhorn and Lorenz [22] phenological scale. The use of herbicide (glyphosate 2.2 g e a $ha^{-1}$) causes the lack of cover vegetation, this system is characterized by plants on bare soil [23]. The application was carried out during three stages, the first in July, the second in stage 17 pre-flowering and the last in the veraison, corresponding to phenological stage 35 [22] using a backpack sprayer (Jacto®) equipped with spout spray tip, 110M303, calibrated to apply 350 L $ha^{-1}$ of herbicide mixture. The herbicide was applied in a width of 1 meter, along the plant rows. Grapes were harvested at the maturation stage, with 23 °Brix during 2014/15 crop and 22 °Brix in 2015/16.

Evaluated phytotechnical variables were fresh cluster (g) and 250 berries (g) weight using a precision balance, yield, production, Ravaz index, leaf surface, percentage of openings, number of leaf layers, inner clusters, the incidence of *Botrytis cinerea* and of acid rot and phenological appraisals. Production per plant was determined with field electronic scales, multiplying the average number of clusters per plant by the average weight of the cluster and the results were expressed in kg $plant^{-1}$. The yield (kg $ha^{-1}$) was obtained by multiplying yield per plant by plantation density.

Ravaz index was obtained from winter dry pruning weight in relation to production [kg of fruit kg (dry pruning) -1] [24]. This index indicates the vigor of the vine, where values between 4 and 7 for *V. vinifera* cultivars inform that there is plant balance. Indices higher than seven indicate overproduction; and, values below four, too much vine vigor.

Percentage of openings (voids), number of leaf layers, percentage of leaves and inner cluster were determined by the Point Quadrat Method [25] during the onset of the grape maturation. The readings were performed perpendicularly in the espalier production region, inserting a rod that simulated the beam of light, from the outside to the foliage interior, and registering which organ of the plant the tip of the stem touched, leaf or cluster or opening. The ideal values are 20 to 40% for opening percentage; <1.5 for leaf layer number; <10% for inner leaves; and <40% for inner clusters.

Potential exposable leaf surface (ELSp) was determined according to Carbonneau [26], and expressed in $m^2$ per plant and hectare.

The incidences of *Botrytis cinerea* and acid rot were obtained through visual evaluation, and presence or absence of disease symptoms was verified. Evaluations were carried out on all clusters presents in two plants per plot. The incidence was calculated by clusters percentage that presented at least one lesion in relation to the total number of clusters.

Phenological evaluations started during pruning and continued until fruits were harvested. Evaluations were based on weekly visual observations using the Eichhorn and Lorenz [22] scale. Based on the phenological stages, the time when the structures reached 50% of the bud stage (stage 05), 50% of full bloom (stage 23), 50% of maturation onset (stage 35) and harvesting (stage 38) was calculated.

The grape composition variables evaluated were pH, soluble solids, and titratable acidity. pH was determined with a Quimis® bench pH meter (model Q400AS, São Paulo, Brazil). Soluble solids content (SS) was quantified with a temperature-compensated digital refractometer (Atago® Palette model, Japan), and the results were expressed in ºBrix. For titratable acidity (TA), 10 ml of the grape must was added to 90 ml of distilled water. Sample titration was done using a digital burette (Vittab®), containing sodium hydroxide solution (0.1 N) until reaching pH 7 and expressed in milliequivalents per liter (meq $L^{-1}$).

In addition to these basic assessments, the density (g $ml^{-1}$) and the contents of tartaric acid (g $L^{-1}$), malic acid (g $L^{-1}$), citric acid (g $L^{-1}$), gluconic acid (g $L^{-1}$), reducing sugars (g $L^{-1}$), glucose (g $L^{-1}$), fructose (g $L^{-1}$), ammonia (mg $L^{-1}$) and potassium (mg $L^{-1}$) were quantified, and these evaluations were carried out using the WineScan® $SO_2$ equipment (FOSS, Denmark) and FOSS software integrator version 1.6.0 (FOSS, Denmark).

Polyphenolic potential variables as total and extractable anthocyanins, total polyphenols index, cell maturation index (CMI), and tannins in the grapes skin were evaluated. These analyses were carried out at Post-Harvest Physiology and Technology of Fruits laboratory, Department of Agroindustrial Science and Technology, Federal University of Pelotas. For phenolic compounds evaluation, the methods by Laffort Oenologie [27], Glories and Augustin [28] and Gaulejac et al. [29] were used. The values obtained from samples ApH1.0 represented the total potential of anthocyanins and ApH3.2 the extractable anthocyanins, results were expressed as mg $L^{-1}$. Total polyphenol index was determined by the ApH3.2 solution with a dilution of 1/100 and a reading of 280 nm. From these data, it was possible to estimate cell maturation index (CMI) and tannin concentration (g $L^{-1}$) in the skins according to González-Neves et al. [30]. The readings were performed using Instrutherm UV-2000A spectrophotometer (Instrutherm, Brazil).

Data obtained in each crop (2014/15 and 2015/16) were analyzed separately for normality by Shapiro Wilk test; homoscedasticity by Hartley test; and graphic analysis was used for residue independence. Subsequently, data were submitted to analysis of variance through the F test ($p \leq 0.05$). Being statistically significant, the effect of the permanent resident vegetation cover was compared by the t test ($p \leq 0.05$).

Subsequently, a joint analysis was carried out with all the determinations, making it possible to compare the effect of resident vegetation cover in each crop and also to compare production crops (2014/15 and 2015/16) by multivariate analysis using the main components method. The presence of correlations between the dependent variables on the study was analyzed using the Pearson correlation coefficient (r).

## 3. RESULTS AND DISCUSSION

Phytotechnological variables as fresh cluster weight, production, yield, Ravaz index, leaf surface expressed in $m^2$ per plant and ha and inner clusters during 2014/15 crop presented higher values in the presence of herbicide application. In others phytotechnical evaluations during this crop, no significance was verified on the effect of permanent resident vegetation cover. On the other hand, there was no significance on the effect of resident vegetation cover during 2015/16 crop either, for all phytotechnical parameters (Table 2).

Regarding all physicochemical variables, both 2014/15 and 2015/16 crops, no significant results were observed on the effect of permanent resident vegetation cover, except for pH in the productive crop of 2014/15, in which 'Tannat' grapes presented higher pH in the presence of vegetation cover (Table 3).

In both crops and for all polyphenolic potential variables, no significance was verified on the effect of permanent resident vegetation cover except for phenolic compounds in the 2015/16 crop, whose grapes presented a greater contribution of total polyphenols in the presence of the herbicide (Table 4).

Related to the treatment factor studied, the data matrix of the analyzed variables consisted of 31 dependent variables among which 13 were phytotechnical, 13 physico-chemical and 5 polyphenolic potential. These data were submitted to PCA in order to reduce the number of descriptors associated to the data set and at the same time preserve most of the variability. In order to explain the distribution of the groups, it was necessary to reduce the number of the main components as a function of the amount of high and/or medium correlations between dependent variables, which is changeable according to the studied population [29]. Therefore, in accordance to Jolliffe [31] rule, only the first two (orthogonal variables) PCs were used in the analyses because they included 70% of the variation, both in the comparison of the treatment factor (vegetation cover) in each production crop (2014/15 and 2015/16) as well as in differences verification between crops (Fig. 3C).

During 2014/15 production crop, a new set of five orthogonal variables (PCs) was generated by PCA, where the first major component (PC1) had the highest eigenvalue of 14.92 and represented 49.73% of the variability in the data set. The second PC had an eigenvalue of 6.38, and was responsible for 21.26% of data variation. The remaining three PCs (PC3, PC4 and PC5), were generated progressively, produced smaller eigenvalues and did not significantly explain the data variability. The first two main components explained a large proportion of total variation, that is, 70.99%, which allowed the plotting of scores and component loads referring to the treatment factor levels studied [with permanent resident vegetation cover (VC) and herbicide usage (HB)] (Fig. 3A). The formation of distinct groups was verified, showing the differentiation between treatment factor levels as a function of dependent variables evaluated. The eigenvectors that correspond to the main component 1 and are a result of original variables loading on this component were analyzed. They represent a relative importance measure of each variable, and among them gluconic acid (0.25) and fresh cluster weight (0.24) are highlighted. Regarding PC2, the percentage of openings (-0.31), number of leaf layers (0.28), fresh weight of 250 berries (0.27), extractable anthocyanins (0.26), yield (0.25) and skin tannin (0.25) also contributed to this differentiation.

**Table 2. Chemical and physical soil characteristics after the experiment was completed**

| Sample[1/] | Profile (cm) | pH water 1:1 | Ca | Mg | Al[1/] | H+Al[3/] | $CTC_{effective}$ | $CTC_{pH7}$ | K | Saturation (%) Al | Saturation (%) Bases | Index SMP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | ---------- | ---------- | ---------- | ---------- | $cmol_c/dm^3$ | ---------- | | | | |
| VC | 0-20 | 5,8 | 4,9 | 1,3 | 0,1 | 2,2 | 6,7 | 8,8 | 0,36 | 1,5 | 75 | 6,6 |
| | 20-40 | 5,3 | 3,5 | 1,4 | 0,2 | 2,8 | 5,3 | 7,9 | 0,17 | 3,8 | 64 | 6,4 |
| HB | 0-20 | 6,1 | 5,4 | 1,5 | 0,0 | 2,0 | 7,2 | 9,2 | 0,28 | 0,0 | 78 | 6,7 |
| | 20-40 | 5,2 | 3,3 | 1,1 | 0,2 | 3,5 | 4,0 | 8,2 | 0,31 | 4,1 | 58 | 6,2 |

| Sample | Profile (cm) | M.O. | Clay | Silite | Sand | S | P-Mehlich | K | Fe (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | ------- m/v ------ | | | | | ---------- $mg/dm^3$ ------------ | | |
| VC | 0-20 | 1,93 | 23 | 12 | 54 | 11,8 | 60,2 | 130 | 0,13 |
| | 20-40 | 1,52 | 30 | 11 | 68 | 14,4 | 11,4 | 65 | 0,13 |
| HB | 0-20 | 2,35 | 20 | 11 | 69 | 10,7 | 40,9 | 110 | 0,14 |
| | 20-40 | 1,66 | 30 | 11 | 68 | 14,5 | 25,0 | 121 | 0,13 |

| Sample | Profile (cm) | Cu | Zn | B | Mn | Na | Molar ratios Ca/Mg | Molar ratios Ca/K | Molar ratios Mg/K |
|---|---|---|---|---|---|---|---|---|---|
| | | ---------- | ---------- | $mg/dm^3$ | ---------- | ---------- | | | |
| VC | 0-20 | 99,2 | 9,5 | -- | 54 | 7 | 3,77 | 13,61 | 3,61 |
| | 20-40 | 21,1 | 3,3 | -- | 66 | 4 | 2,50 | 20,59 | 8,24 |
| HB | 0-20 | 174,4 | 22,8 | -- | 44 | 4 | 3,60 | 19,29 | 5,36 |
| | 20-40 | 30,1 | 4,3 | -- | 103 | 3 | 3,00 | 10,65 | 3,55 |

*[1/] Extractor Mehlich-1. [2/] Extrator KCl 1.0 mol $L^{-1}$. [3/] Extractor $Ca(OAc)_2$ 0.5 mol $L^{-1}$, pH 7.0*

**Table 3. Phytotechnical variables of 'Tannat' grapes as a function of the permanent resident vegetation cover (VC) and herbicide use (HB) in the 2014/15 and 2015/16 productive crops. Bagé-RS**

| Phytotechnical variables | Productive crops | | | |
|---|---|---|---|---|
| | 2014/15 | | 2015/16 | |
| | VC | HB | VC | HB |
| Fresh Weight of cluster (g) | 222.05±9.49b[1/] | 292.40±12.42a | 267.88±22.75$^{NS}$ | 267.83±1.09 |
| Fresh Weight of 250 berries (g) | 494.75±16.23$^{NS}$ | 484.00±22.55 | 477.25±8.14$^{NS}$ | 452.25±12.68 |
| Production (Kg planta$^{-1}$) | 4.50±0.07b | 5.45±0.16a | 4.49±0.66$^{NS}$ | 5.89±0.65 |
| Yield (Kg ha$^{-1}$) | 12.463±21.15b | 13.426±15.35a | 12.060±22.89$^{NS}$ | 12.332±11.12 |
| Ravaz Index | 4.94±0.25b | 6.74±0.10a | 5.37±0.21$^{NS}$ | 5.36±0.80 |
| Leaf Surface (m$^2$ planta$^{-1}$) | 2.13±0.04b | 2.28±0.04a | 2.13±0.04$^{NS}$ | 2.28±0.03 |
| Leaf Surface (m$^2$ ha$^{-1}$) | 5.378±102.28b | 5.768±104.49a | 5.375±99.62$^{NS}$ | 5.749±67.93 |
| Openings (%) | 12.82±1.28$^{NS}$ | 11.61±1.53 | 10.86±2.40$^{NS}$ | 8.13±0.44 |
| Leaf layer number | 0.77±0.01$^{NS}$ | 1.08±0.08 | 1.94±0.03$^{NS}$ | 1.88±0.63 |
| Inner leaves (%) | 9.44±2.00$^{NS}$ | 12.80±9.45 | 21.64±3.23$^{NS}$ | 32.05±0.64 |
| Inner clusters (%) | 34.95±3.78b | 53.57±0.79a | 69.52±3.51$^{NS}$ | 67.96±4.38 |
| *Botrytis cinerea* (%) | 1.29±0.39$^{NS}$ | 1.21±0.31 | 0.00±0.00$^{NS}$ | 0.28±0.18 |
| Acid rot (%) | nd | nd | 2.65±0.86$^{NS}$ | 2.56±1.22 |

*[1/] Means (of four determinations ± standard error) accompanied by the same letter in the row do not differ among themselves by the t test (p≤0.05) comparing VC and HB in each productive crop. nd: not determined. $^{NS}$: Not significant by the F test (p≤0.05) of analysis of variance*

**Table 4. Physicochemical variables of 'Tannat' grapes as function of the permanent resident vegetation cover in the productive crops of 2014/15 and 2015/16. Bagé-RS**

| Physicochemical variables | Production crops | | | |
|---|---|---|---|---|
| | 2014/15 | | 2015/16 | |
| | VC | HB | VC | HB |
| pH | 3.62±0.01a[1/] | 3.56±0.02b | 3.37±0.03$^{NS}$ | 3.36±0.04 |
| Soluble Solids (°Brix) | 23.57±0.27$^{NS}$ | 23.05±0.51 | 23.07±0.56$^{NS}$ | 22.16±0.84 |
| Titratable acidity (meq L$^{-1}$) | 110.87±4.94$^{NS}$ | 119.25±4.37 | 111.75±4.70$^{NS}$ | 124.00±5.11 |
| Density (g ml$^{-1}$) | 1.09±0.00$^{NS}$ | 1.08±0.00 | 1.09±0.00$^{NS}$ | 1.09±0.00 |
| Tartaric acid (g L$^{-1}$) | 2.63±0.03$^{NS}$ | 2.63±0.03 | 3.68±0.11$^{NS}$ | 3.82±0.30 |
| Malic acid (g L$^{-1}$) | 3.57±0.18$^{NS}$ | 3.90±0.26 | 3.85±0.27$^{NS}$ | 4.15±0.18 |
| Citric acid (g L$^{-1}$) | 0.40±0.00$^{NS}$ | 0.43±0.03 | 0.50±0.00$^{NS}$ | 0.52±0.02 |
| Gluconic acid (g L$^{-1}$) | 0.30±0.06$^{NS}$ | 0.73±0.14 | 0.20±0.10$^{NS}$ | 0.15±0.03 |
| Reducing sugars (g L$^{-1}$) | 221.17±4.78$^{NS}$ | 206.53±6.11 | 218.32±5.08$^{NS}$ | 218.47±5.08 |
| Glucose (g L$^{-1}$) | 106.60±2.15$^{NS}$ | 100.07±2.99 | 107.87±2.29$^{NS}$ | 107.60±2.40 |
| Fructose (g L$^{-1}$) | 108.20±2.20$^{NS}$ | 100.73±2.92 | 105.45±2.64$^{NS}$ | 105.20±2.33 |
| Ammonia (mg L$^{-1}$) | 44.67±5.90$^{NS}$ | 47.00±8.50 | 68.00±6.57$^{NS}$ | 73.00±1.91 |
| Potassium (mg L$^{-1}$) | 794.00±86.43$^{NS}$ | 1.048±28.16 | 359.50±14.68$^{NS}$ | 466.50±15.33 |

*[1/] Means (of four determinations ± standard error) accompanied by the same letter in the row do not differ among themselves by the t test (p≤0.05) comparing VC and HB in each productive crop. $^{NS}$: not-significant by the F test (p≤0.05) of analysis of variance*

The first two main components in the 2015/16 crop explained 84.08% of the total variation, where PC1 was responsible for 50.95% with the highest eigenvalue (15.79) and PC2 represented 33.13%, with an eigenvalue of 10.27 (Fig. 3B). Only two PCs were used in the additional analyses, the other PC generated (PC3) did not significantly explain the data variability (15.92%) and was excluded from the analysis [31]. The use of vegetation cover showed the same behavior observed in the previous crop (2014/15), that is, there was differentiation between permanent resident vegetation cover (VC) and the presence of the herbicide (HB), from distinct groups formation (Fig. 3B). However, the dependent variables that allowed this differentiation were different. Analyzing the eigenvectors corresponding to PC1, malic acid

(0.25) and percentage of inner leaves (0.25) stood out, and in PC2, the pH (0.30), leaf layer number (0.28), acid rot (0.28) and gluconic acid (-0.26) stood out, with all contributing their loads to differentiate vegetation cover usage.

The group formed by herbicide (HB) characterized the highest values for malic acid and inner leaves. However, the highest leaf layer values were verified for permanent resident vegetation cover (VC) (Tables 2, 3 and Fig. 3B).

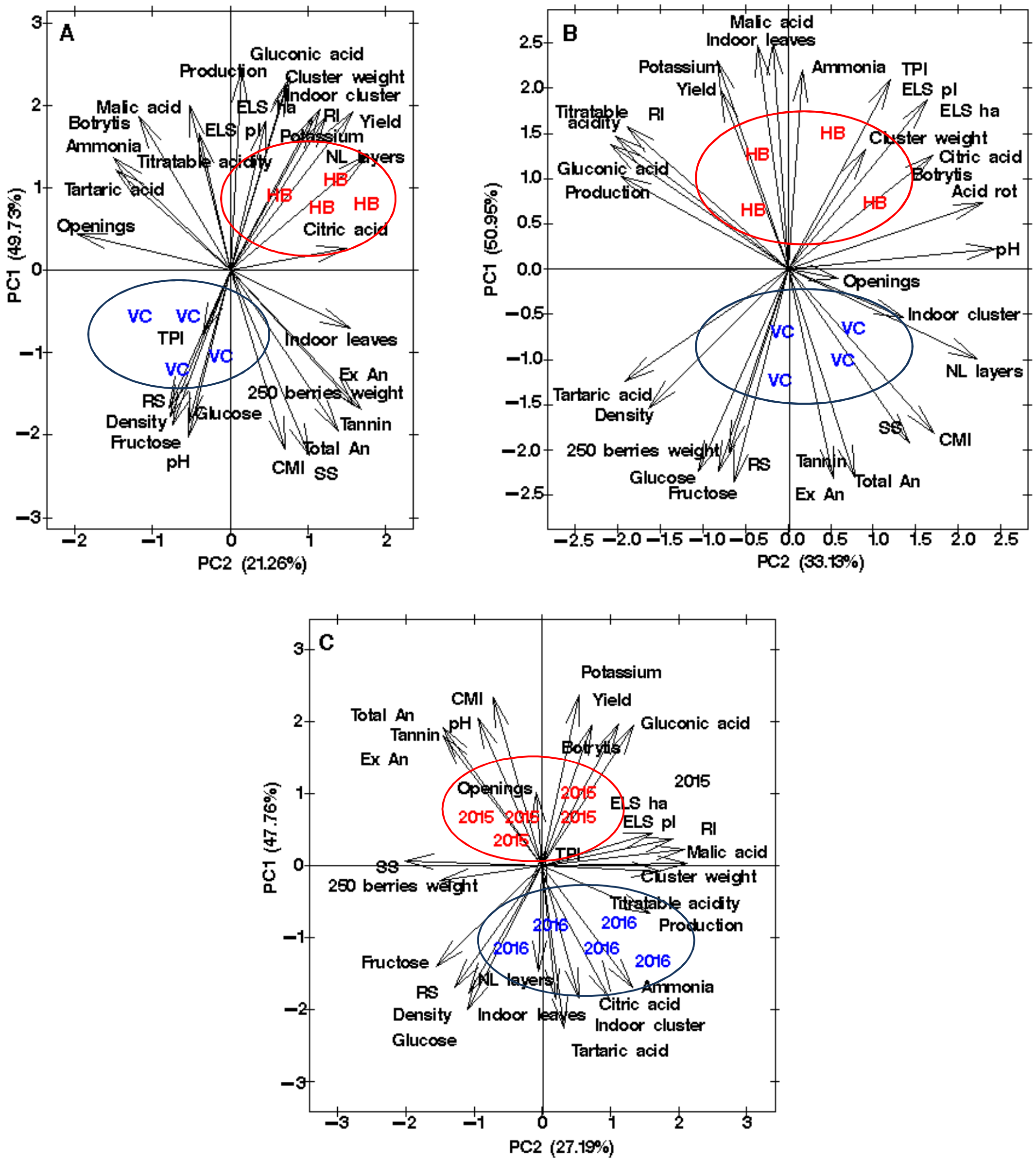


**Fig. 3. Plot of the PC1-PC2 scores and loads for the variables analyzed separately in 2014/15 (A) and 2015/16 (B) considering the 'Tannat' grape variety submitted to permanent resident vegetation cover (VC) and herbicide (HB), and jointly (C) showing the separation of production crops 2014/15 (2015) and 2015/16 (2016), in Bagé, RS. RI: Ravaz index; ELS pl and ha: exposable leaf surface expressed in $m^2$ $plant^{-1}$ and $ha^{-1}$; NL layers: number of leaf layers; SS: soluble solids; RS: reducing sugars; Total An: total anthocyanins; Ex An: extractable anthocyanins; TPI: total polyphenols index; CMI: cell maturation index**

**Table 5. Polyphenolic potential variables of 'Tannat' grapes as a function of the permanent resident vegetation cover in the productive crops of 2014/15 and 2015/16. Bagé-RS**

| Variables of polyphenolic potential | Productive crops | | | |
|---|---|---|---|---|
| | 2014/15 | | 2015/16 | |
| | VC | HB | VC | HB |
| Total anthocyanins (mg $L^{-1}$) | 2.034±79.91$^{NS}$ | 1.894±221.83 | 1.278±164.46$^{NS}$ | 1.122±136.56 |
| Extractable anthocyanins (mg $L^{-1}$) | 638.24±26.99$^{NS}$ | 620.88±49.96 | 501.98±42.22$^{NS}$ | 524.94±83.23 |
| Total polyphenol content (IPT) | 23.17±5.16$^{NS}$ | 19.17±1.08 | 14.00±2.90b [1/] | 22.25±1.49a |
| Cell Maturation Index (IMC) | 91.50±0.31$^{NS}$ | 89.19±2.01 | 59.78±2.48$^{NS}$ | 52.95±6.19 |
| Skin Tanin (g $L^{-1}$) | 25.53±1.08$^{NS}$ | 24.83±1.99 | 20.08±1.69$^{NS}$ | 21.00±3.33 |

*[1/] Means (of four determinations ± standard error) accompanied by the same letter in the row do not differ among themselves by the t test (p≤0.05) comparing VC and HB in each productive crop. $^{NS}$: Not significant by the F test (p≤0.05) of the analysis of variance*

When the analysis was carried out jointly, it was observed that the two productive crops studied presented a differentiated behavior regarding the characteristics evaluated; this is visible in Fig. 3C. The first two main components accounted for 74.95% of the total variation, where PC1 accounted for 47.76% with an eigenvalue of 14.33 and PC2 characterized 27.19% of the differentiation with a minor eigenvalue (8.16). The other PCs (PC3 to PC8) did not contribute significantly to the differentiation. The variables that were determinant for groups separation with their respective eigenvectors were potassium (0.28), CMI (0.28) and tartaric acid (-0.27) for PC1, and malic acid (0.30), weight (0.29), soluble solids (-0.28) and Ravaz index (0.27) in PC2. In the 2014/15 crop, higher values were observed for potassium, CMI, and Ravaz index, and in 2015/16 the highest values occurred mainly for tartaric and malic acid (Tables 2, 3, 4 and Fig. 3C).

The variables ELSp (Potential exposable leaf surface) and leaf layers had a grouping in herbicide usage, the same for variables production, yield and Ravaz index during 2014/2015 crop. This shows a greater use of both water and minerals by the plant, since it does not have the competition of the vegetation cover. On the other hand, a grouping of unwanted variables like gluconic acid (Botrytis) and potassium also appears.

Vigor excess can lead to increased shading inside the espalier which leads to a rot and potassium increments in the clusters. Poor photosynthetic activity by shaded leaves raises potassium translocation from these leaves to the berries [32].

The variable ammonia was grouped together with the variable *Botrytis*. The ammoniacal content in the berry is closely correlated with grapes susceptibility to *Botrytis cinerea* [33]. It is the use of vegetation cover that causes available nitrogen reduction to the vineyard by immobilizing part of the soil nitrogen, taking into account that the highest percentage of permanent resident vegetation cover species are grasses.

On the other hand, a production increase, mainly when using the Guyot pruning system, tends to cause clusters overlapping which hinders their aeration and illumination, leading to cluster rot and potassium increasing.

This is how positive correlations occurred between cluster weight and gluconic acid (r = 0.91, p = 0.01), cluster weight and potassium (r = 0.88, p = 0.02), yield and potassium (0.83, p = 0.04), and exposable leaf surface potential and gluconic acid (r = 0.82, p = 0.05). It is worth mentioning that gluconic acid occurs in botrytised grapes or with *Aspergilus* sp., and is used as an indicator of rot attacks [32,33].

Similarly, the use of spontaneous vegetation cover during the 2014/2015 crop, grouped variables associated to quality, such as reducing sugars, phenols, skin tannins and total extractable anthocyanins. Tesic et al. [34] and Reynier [35] found that the use of permanent plant cover decreases vigor and yields with a sugar increase in the berry, mainly in dry summers. Afonso et al. [1] also found a yield reduction using permanent resident vegetation with Alvarinho cultivar in the *Vinhos Verdes* region, due to the cluster weight decrease as well as a reduction in vigor, attributing this phenomenon to a plant self-compensation to maintain a source-sink balance.

During 2015/2016 crop, a grouping of production, yield and cluster weight was maintained for herbicide use. Likewise, the variables associated with vigor such as ELSp were maintained for the in-row herbicide use. The variables potassium, *Botrytis* and gluconic acid were grouped together for this treatment. Positive correlations were found between potassium and yield (r = 0.89, p = 0.02), potassium and inner leaves (r = 0.94, p = 005), inner leaves and ELSp.ha$^{-1}$ (r = 0.84, p = 0.03). This is consistent with Coniberti et al. [17] research, where they conclude that the sun exposure inside the foliage is a determining factor for lowering the potassium content in the berries and later in the wort.

Similar to the previous crop during in-row vegetation cover treatment, there was components grouping related to the quality such as reducing sugars, density, skin tannin, total and extractable anthocyanins, coinciding with that published by Fregoni [15], Tesic et al. [34] and Coniberti et al. [17].

The proximity of the CMI and sugar accumulation variables (density, glucose, fructose and reducing sugars) as well as titratable acidity separation showed that the permanent vegetation cover accelerated grapes maturation for the two years. This is also shown by the distancing of vegetation cover usage with the malic and tartaric acid variables. Malic acid, in particular, is metabolized in the berry more rapidly during the last maturation phase in relation to tartaric acid, because it is less resistant to oxidative respiration [33]. In addition, acids are produced in photosynthetically active organs and green berries, then an increase in ELSp may have induced these higher values for herbicide treatment.

In relation to the year effect, the 2015/2016 crop, behavior with higher water deficit in the period from maturation onset to harvest (January and February) (Fig. 2B), the variables were in accordance to the logic of a drier crop, where the sugar accumulation variables were concentrated in this crop (Fig. 3C).

The variables *Botrytis*, gluconic acid and potential exposable leaf surface are grouped in the crop 2014/2015, a period with higher number of precipitations in the maturation period, (Fig. 2A) showing higher production and yield, greater vigor and a greater susceptibility to cluster diseases.

Working with in-row plant cover of ‘Tannat’ cultivar in Uruguay, Coniberti et al. [17] concluded that the vigor decrease by the vegetation cover usage lowers the incidence of *Botrytis,* because of the vigor decrease. Without this, there would be a yield reduction.

The 2014/2015 crop also shows a higher concentration of total and extractable anthocyanins. Anthocyanins reach their maximum content on ripening, their synthesis has an optimal range of action between 17 and 25°C, but when it exceeds 35°C, an inhibition of this synthesis occurs or an increase of its degradation [35]. The 2015/2016 crop has notably higher peak temperatures in the months of January and February which reduced anthocyanin content compared to the previous crop.

All these results shows that for the soil type where the experiment was implanted, the use of permanent in-row vegetation cover is far from causing any harm to the grapes, it demonstrated signs of quality enhancement, improving the canopy structure and crop relation, specifically for the 2014/2015 and 2015/2016 crops.

## 4. CONCLUSION

The use of permanent resident in-row vegetation cover is able to control the vigor and elevate quality parameters in grapes. Particularly in the deep soils of the *Campanha* region, is a technique that besides promoting sustainability in its conservation, can reduce costs of herbicides usage, green pruning, and mainly of thinning when aiming to increase the quality.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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