The effect of the nature of the soil amendments on the main aromatic compounds in *Vanilla fragrans* beans

Éric Odoux[a*]
Sarah Languille[b]
Jean-Patrice Leblé[b]
Aïdée Lombard[b]

[a] Cirad-Flhor,
BP 5035,
34032 Montpellier cedex 01,
France
(eric.odoux@cirad.fr)

[b] Laboratoire d'analyses chromatographiques du Cirad,
Centre de la Réunion,
Maison régionale
des sciences et technologies,
100, route de la Rivière-des-Pluies,
97490 Sainte-Clotilde,
île de la Réunion, France

** Correspondence and reprints*

Received 16 February 1999
Accepted 18 June 1999

Fruits, 2000, vol. 55, p. 63–71

Resumen Español, p. 71

The effect of the nature of the soil amendments on the main aromatic compounds in *Vanilla fragrans* beans.

Abstract — Introduction. Given the economic context in Réunion and the lack of reliable information on the fertilization of vanilla, a study was carried out to compare the effect of different substrates available in the island on the growth of vines and bean yields. Highly significant differences of vine growth and flowering induction between treatments were highlighted. The aim of the work presented was to study the effect of substrate composition on the main aromatic compounds in the beans grown. **Materials and methods**. Eight soil amendments were used to test the vanilla (*Vanilla fragrans*) production on soil-less substrates, grown under shade houses. The beans were picked at the harvest stage (yellow colour). Beans of vanilla grown using traditional cultivation methods were studied as control. After extraction, four aromatic compounds (vanillin, vanillic acid, 4-hydroxybenzoic acid and 4-hydroxybenzaldehyde) were analysed with a HPLC method. **Results and discussion**. The bean aromatic contents of the eight amendments used appeared not to be significantly different. However, vanilla beans grown traditionally under wood appeared to display substantial qualitative differences of vanillin and vanillic acid contents in comparison with those of the experimental shade house plot. **Conclusion**. A next study would have to be undertaken to identify the parameters responsible for the differences observed between these two types of vanilla cultivation. © Éditions scientifiques et médicales Elsevier SAS

Vanilla (spice) / organic amendments / aromatic compounds / France (Réunion)

Effet de l'amendement du sol sur les principaux composés aromatiques de la gousse de vanille.

Résumé — Introduction. Étant donné le contexte économique de l'île de la Réunion et le manque d'informations fiables sur la fertilisation de la vanille, une étude a été effectuée pour comparer l'effet, sur la croissance des lianes et les rendements en gousses, de différents types d'amendement disponibles sur l'île. Des différences hautement significatives portant sur la croissance des lianes et l'induction florale ont été mises en évidence entre les traitements. Le but du travail présenté a été d'étudier l'effet de la composition de différents substrats de culture sur les principaux composés aromatiques des gousses. **Matériel et méthodes**. Huit types d'amendement ont été testés vis-à-vis de la production de vanille (*Vanilla fragrans*) cultivée en hors sol sous ombrières. Les gousses ont été cueillies au stade de récolte (couleur jaune). Des gousses de vanille cultivée de façon traditionnelle ont été utilisées comme référence. Après extraction, quatre composés aromatiques (vanilline, acide vanillique, acide 4-hydroxybenzoic et 4-hydroxybenzaldehyde) ont été analysés par HPLC. **Résultats et discussion**. Les teneurs aromatiques des gousses des huit types d'amendements utilisés n'ont pas présenté de différences significatives. Cependant, les gousses de vanille cultivée de façon traditionnelle en sous-bois ont semblé montrer de réelles différences qualitatives de teneurs en vanilline et acide vanilliques par comparaison avec celles des parcelles expérimentales sous ombrières. **Conclusion**. Une prochaine expérimentation devra être entreprise pour identifier les paramètres responsables des différences observées entre ces deux types de culture de vanille. © Éditions scientifiques et médicales Elsevier SAS

vanille / amendement organique / composé aromatique / France (Réunion)

1. introduction

The world production of cured vanilla is approximately 2 000 t per year, with production shared between Madagascar, Indonesia and, to a lesser extent, the Comoros [1]. China should shortly become the fourth country on the market, with a considerable production potential to judge by the extent of soil-less culture established [2]. Production costs are very low in these countries in comparison with those in Réunion. Given the very competitive economic context, Réunion vanilla planters have applied an intensification programme for vanilla *(Vanilla fragrans)* since 1994, based on soil-less culture establishment with artificial shade and better conduct of soil amendments, with the aim of improving the productivity of vanilla vines. Even if the industrial vanilla market seems compromised for Réunion, the reconquest of the local market by producing high-quality household vanilla could be a significant issue, if production costs can be reduced.

Not many works have been published on vanilla, and many of them concern the physicochemical analysis of black vanilla beans and their extracts [3–5] or the distinction between natural and synthetic vanillin [6–8]. The agronomic and physiological aspects of the crop have not received as much attention [9–12].

Given the economic context in Réunion and the lack of reliable information on the fertilization of vanilla, Cirad[1] set up a trial to compare the effect of different substrates available in the island on the growth of the vines and bean yields. The study has made it possible to show highly significant differences of vine growth and flowering induction between treatments [13, 14]. It was therefore interesting to verify whether the nature of the substrates also affects the biosynthesis of aromatic compounds, especially as it was considered locally that organic matter improved the vanillin content of beans. The purpose of this work was to show the effect of substrate nature on the main aromatic compounds in the beans grown.

[1] Cirad, *Centre de coopération international en recherche agronomique pour le développement.*

2. materials and methods

2.1. plant material

The vanilla vines *(Vanilla fragrans)* used in the study were those commonly grown by Réunion farmers. They are not of a particular variety or cultivar as there is no local distinction of this kind. The plant material was supplied by Provanille, a local organization for training the vanilla growers.

2.2. agronomic conditions

Two shade houses were constructed to create a test block for the comparison of eight treatments characterized by various cultivation substrates, with eight repetitions (*table I*).

These substrates were originally chosen [13] to measure the nutritive requirements of the vanilla plant and, in particular, the possible importance of decomposing organic matter and fertilizer application and the effect of the more or less ligneous nature of the organic matter; too, they were chosen to study the role of symbiotic fungi in the decomposition of organic matter.

The shade houses were tunnel structures covered with 60 % shade cloth. T-shaped wooden stakes were used at a rate of one per three cuttings. The cuttings were 1 m long, chosen at random and planted in May 1994. Mist watering conditions were optimized for satisfactory moistening of the substrates and for the best possible limiting of known fungal diseases. At weekly intervals, two fungicides (fosetyl and benomyl) were applied alternately throughout the summer from October to April. A Basacote™ 6 month (14–10–13) slow-release fertilizer was applied at 100 g per plot in June and February.

The flowers were pollinated manually (ten flowers per inflorescence), in the morning, during the flowering period from September to December.

The beans were picked at the harvest stage (yellow color) between May and August and frozen at –18 °C to await analysis.

A batch of some 50 vanilla beans from the Sainte-Suzanne region, north-east of the

Table I.
The composition of the various substrates tested for the soil-less vanilla vine cultivation in Réunion.

Treatment	Volcanic slag ratio	Other substrate component
T1	100 %	–
T2	100 %	Fertilizer Basacote™
T3	1/3	2/3 sugar cane *(Saccharum* spp.*)* bagasse compost
T4	1/3	2/3 Japanese cedar *(Cryptomeria japonica)* compost
T5	1/3	2/3 casuarina *(Casuarina equisetifolia)* compost
T6	1/3	2/3 residual sludge from the town Le Port, Réunion, composted with *Cryptomeria japonica* residues
T7	1/3	2/3 treated maritime pine compost *(Pinus maritimus)*
T8	1/3	2/3 bagasse compost + slow-release fertilizer Basacote™

island, was also obtained. This vanilla was grown using traditional methods under natural shade on live prop-plants and with no added substrate or fertilizer. It was used to compare the levels of the natural bean aromatic compounds with those of the vanilla grown experimentally on vines in shade houses.

2.3. extraction of aromatic compounds

The green beans were cut into pieces and ground. A 15 g sample of the ground preparation was weighed out, 20 mL distilled water added and the mixture ground for approximately 1 min. The preparation was recovered and made up to 75 g total mass with distilled water. The mixture was used for enzymatic hydrolysis of the glycosylated precursors using a β-glucosidase (Fluka, ref. 49290) by means of a procedure derived from those of Brunerie [15] and of Brodelius [16]. Incubation was performed in a water bath at 35 °C, pH 5, for 1 h, and agitated. The medium contained 50 mg enzyme, i.e., 20 $U \cdot g^{-1}$ green bean, enabling total hydrolysis of the glycosylated precursors. The mixture was filtered on paper and 500 µL filtrate was diluted to 1/50 in the mobile phase and, finally, filtered again at 0.45 µm before HPLC analysis.

2.4. HPLC conditions

Analyses were performed with a Spectra Series P100 apparatus (Thermo Separation Products) fitted with a 20 µL sampler and a UV detector set at 254 nm. A Licrospher® 100 (Merck) RP18e (5 µm) separation column 250 mm long and 4 mm in diameter was used; the temperature of the column was set thermostatically at 30 °C.

The mobile phase was a mixture of 35 % methanol (MeOH) and 65 % phosphoric acid (H_3PO_4) 10^{-2} M. Flow on the column was 0.7 $mL \cdot min^{-1}$, i.e., a pressure of approximately 135 bars.

The compounds were quantified using the external standard technique with a calibration curve. The reference compounds were supplied by Fluka (vanillin, ref. 94750; vanillic acid, ref. 94770; 4-hydroxybenzoic acid, ref. 54630; 4-hydroxybenzaldehyde; ref. 54589). All these compounds were analytical grade.

2.5. determination of dry weight

The determination of dry weight was performed by replacing a 5 g paste after the first grinding of green vanilla, oven-drying the sample at 60 °C for 24 h, then weighing it after drying.

2.6. determination of sample size

A batch of green vanilla beans was collected at random in a local vanilla cooperative and 30 of them were analysed individually using the procedures described above. The quantities of enzyme and water added during the aromatic compound extraction phase were proportional to the weight of the bean examined.

Determination of sample size was performed as follow.

We calculated the standard deviation (s) of this population of 30 individuals for each of the four compounds, and we considered that s is the standard deviation of the whole population.

The standard deviation s_n of the population of n individuals in the sample batch is:

$$s_n = s / \sqrt{n} \qquad (1)$$

The value of s_n must therefore be set in order to determine n, the sample size, for a precision p, in percentage of the mean m, for a given probability. This can be writen:

$$m - pm < m < m + pm \qquad (2)$$

In the case of a Gaussian distribution, we can also write, for a given probability α:

$$m - t_\alpha \cdot s_n < m < m + t_\alpha \cdot s_n \qquad (3)$$

where t_α is given by the Student's tables.

From equations (2) and (3), it comes:

$$pm = t_\alpha \cdot s_n \text{ or } s_n = pm / t_\alpha$$

Reported in equation (1), it comes:

$$n = (t_\alpha \cdot s / pm)^2$$

As the p value cannot be lower to the repeatability of the extraction and the HPLC analysis of the compounds, we calculated this one based on ten extractions using the procedure described. The measured repeatability (coefficient of variation or CV) is respectively 9.4, 10.9, 11.4 and 14.9 for vanillin, vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid. So, p was set at 15 %, which was the least good result, to provide better security in sample size calculation.

For $\alpha = 0.95$ and $n > 30$, t is approximatively equal to 2 and n can be calculated. If n calculated is lower than 30, we used the correspondant t value in the Student's table to recalculate n. Step by step, it is possible to approximate the n value with the correspondant t value.

3. results and discussion

The results obtained from the batch of 30 beans analysed individually (*table II*) show that the minimum number of beans n that should be sampled with a precision of $p = 15$ % varied from 8 to 33 beans according to the compound. The marked variability of vanillic acid concentrations (CV = 37.8 %) and 4-hydroxybenzaldehyde (CV = 42.6 %) in the batch considered will be noted (*table III*). These results were used to set the sampling size at 30 beans per plot when it was possible to achieve satisfactory reliability.

In contrast, with an idea generally accepted by vanilla growers, no correlation was observed between the vanillin content and the bean length (*figure 1*).

The substrate added to the soil strongly affected the number of beans collected (*table III*), confirming previous results concerning the number of inflorescences [13–14].

Table II.
Average concentrations of aromatic compounds in a batch of 30 green vanilla beans analysed individually and the smallest number of beans (n) in the sample for a precision of $p = 15$ %.

Aromatic compound	Concentration (% dry matter)	Standard deviation	Coefficient of variation (%)	n at $p = 15$ %
4-Hydroxybenzoi acid	0.024	0.007	29.2	16
Vanillic acid	0.130	0.050	37.8	26
4-Hydroxybenzaldehyde	0.260	0.110	42.6	33
Vanillin	3.600	0.700	18.7	8

Table III.
Vanilla bean production for each of the eight repetitions of the eight treatments presented in *table I* and used to test different cultivation substrates for vanilla. Each repetition is represented by a plot of three vines.

Treatment	Number of repetitions without beans out of 8	Repetition number with bean production	Number of beans harvested per repetition	Total weight of beans per repetition (g)
T1	8	–	–	–
T2	6	R2	11	96
		R8	23	223
T3	1	R1	26	334
		R2	65	660
		R3	62	575
		R4	98	935
		R5	5	47
		R6	10	141
		R8	20	165
T4	5	R5	4	59
		R6	7	96
		R7	9	60
T5	3	R1	12	101
		R3	14	167
		R5	16	202
		R6	14	268
		R8	21	207
T6	0	R1	44	420
		R2	109	574
		R3	165	1 176
		R4	51	253
		R5	55	776
		R6	28	281
		R7	95	682
		R8	114	725
T7	5	R1	5	64
		R5	2	31
		R8	9	114
T8	0	R1	230	1 474
		R2	319	2 210
		R3	38	365
		R4	210	1 543
		R5	108	1 209
		R6	65	567
		R7	361	2 148
		R8	48	277

The number of 30 beans chosen as sample size was not always attained, as in treatments 1, 2, 4, 5 and 7, for instance. These treatments consisted of vines that had a poor development in the substrates used and whose general appearance (short, thin vines, yellow colour) displayed obvious deficiencies. In contrast, the vines in treatments 3, 6 and 8 were vigorous and dark green, which meant a good development on the substrate considered.

The distribution by class of each compound (*figures 2, 3, 4, 5*) makes it possible to plot a practically symmetrical Gaussian

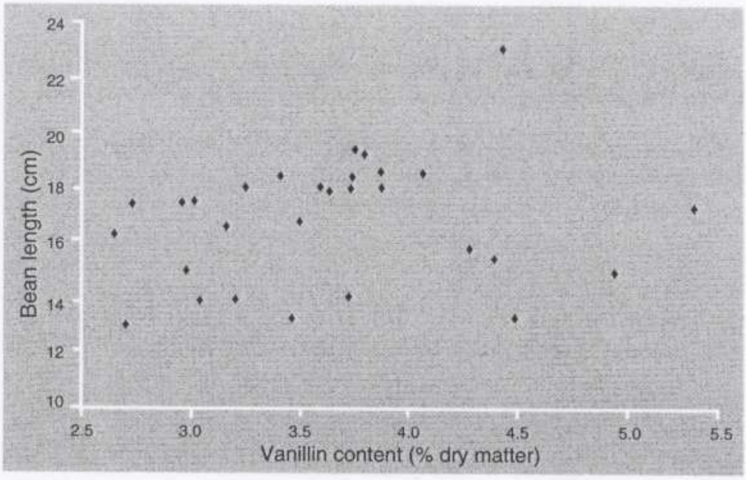
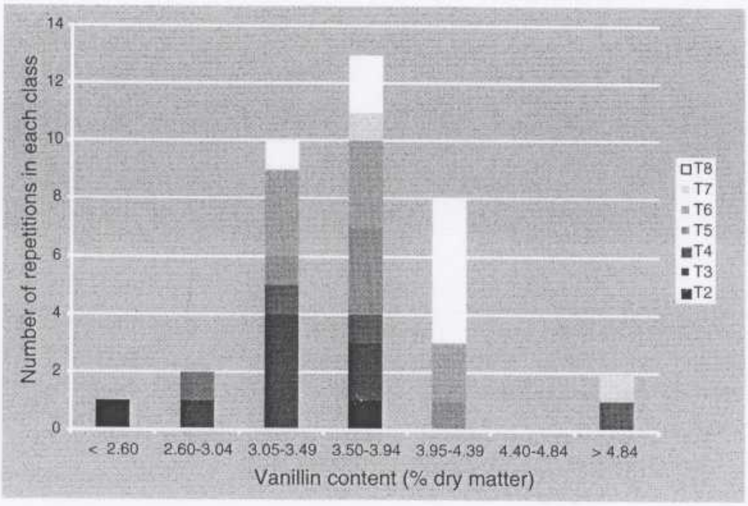


Figure 1.
Relation between vanillin content and bean length.

Figure 2.
Distribution of the repetitions of eight treatments testing the use of different soil amendments for vanilla cultivation, according to classes of bean vanillin content. The treatments T1 to T8 are those presented in *table I*.



curve, and the coefficients of variation (*table IV*) are of the same order of size with that of the repeatability of extraction (15 %). In contrast, the coefficients are lower than those in the batch of 30 beans analysed individually, at least for vanillic acid and 4-hydroxybenzaldehyde compounds (*table II*). It therefore seems that the different plots form a relatively homogeneous group regarding the concentration of aromatic compounds.

Analysis of the distribution of treatments within the classes shows that most of the repetitions (5 out of 8) of treatment 8 (1/3 slag + 2/3 bagasse compost + slow-release fertilizer) produced vanilla beans whose vanillin content was in the 3.95 to 4.40 % concentration class (*figure 2*), higher than average. The repetitions of the other treatments are spread over all the classes. Likewise, the vanillic acid content is higher than average in 6 out of 8 cases in treatment 8 (*figure 3*). A similar remark could be made concerning 4-hydroxybenzaldehyde in treatments 5 and 7 (*figure 4*), but with reserves since few repetitions gave beans and, in addition, in numbers that were much too small to make up a representative sample. Finally, no treatment seemed to show any trend of this type for 4-hydroxybenzoic acid (*figure 5*). These observations confirm the homogeneity of the group of plots. Only treatment 8 seems to stand out slightly from the other treatments in the vanillin and vanillic acid concentrations, but this does not seem to be very significant.

Even if the batch of vanilla grown traditionally in under wood has not been a part of the block trial, it is interesting as the results obtained (*table IV*) confirm a set of analyses performed on black vanilla after preparation (unpublished results). Indeed, although the 4-hydroxybenzoic acid and 4-hydroxybenzaldehyde concentrations were very close to the averages observed in the shade house vanilla, this was not the case for vanillic acid and vanillin, whose concentrations were distinctly lower. It would obviously have been interesting to study several batches of under wood vanilla from different regions and different farmers to confirm or not the differences observed.

4. conclusion

The study set up in Réunion in 1994 to compare the nature of the substrate used in vanilla growing has revealed spectacular agronomic differences [13–14], especially in the induction of flowering. After 3 years of crops, the effects can be observed on the number of beans harvested according to the treatment (although pollination forms a further bias). In contrast, the results presented do not show differences between treatments in the concentration of the four main aromatic compounds in the beans, or the differences observed are of low significance from the scientific point of view (although no reliable scientific verdict supports these results) and even less from the commercial point of view. The nature of the substrate does not therefore seem to affect the biosynthesis of the main aromatic compounds present in the beans although the vanilla vines themselves displayed marked physiological differences in behaviour.

Conversely, vanilla beans grown traditionally in under wood appeared to display substantial qualitative differences in comparison with those of the experimental shade house plot. The differences cannot probably be ascribed to the absence of organic matter or fertilizer. This point deserves to be confirmed for a subsequent attempt to identify the parameters responsible for the differences in the biosynthesis of vanillin and vanillic acid.

However, this type of soil-less cultivation with artificial shade has many advantages in comparison with traditional techniques: earlier flowering, faster vine growth, better yields, less labour time (especially for pollination), the possible extension of production zones, etc.

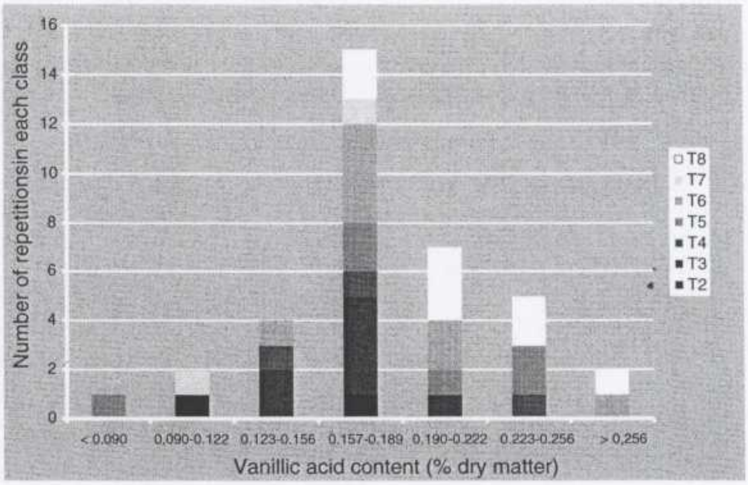
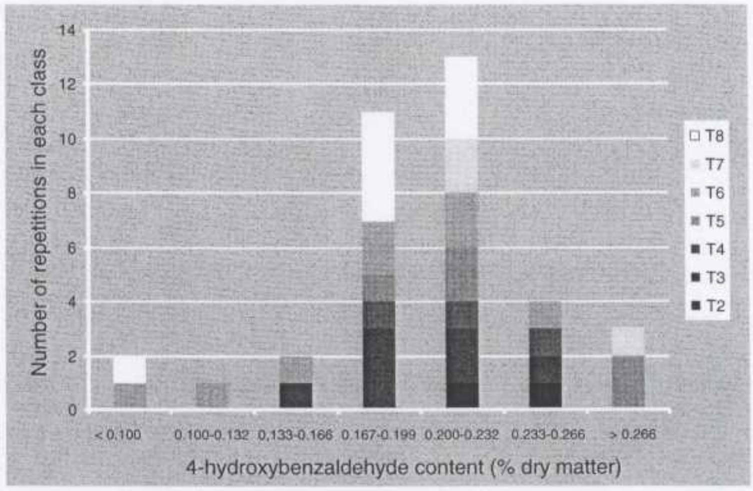


Figure 3.
Distribution of the repetitions of eight treatments testing the use of different soil amendments for vanilla cultivation, according to classes of bean vanillic acid content. The treatments T1 to T8 are those presented in *table I*.

Figure 4.
Distribution of the repetitions of eight treatments testing the use of different soil amendments for vanilla cultivation, according to classes of bean 4-hydroxybenzaldehyde content. The treatments T1 to T8 are those presented in *table I*.



acknowledgments

The authors thank Véronique Lefèvre (*Chambre de Commerce et d'Industrie*, Réunion, France) for having provided the HPLC apparatus required for this work and Bertrand Côme (Provanille company) for providing a sample of green vanilla beans grown in under wood.

references

[1] Blarel B., Dolinsky D., Market imperfections and government failures: the vanilla sector in Madagascar, In: Marketing Africa's high-value foods: comparative experiences of an emergent private sector, Kendall/Hunt Publishing Company, Dubuque, Iowa, 1995.

[2] Basset F., La Chine augmente ses plantations de vanille, Arômes, additifs, ingrédients 8 (1997) 16.

[3] Ranadive A.S., Vanillin and related flavor compounds in vanilla extracts made from beans of various origins, J. Agric. Food Chem. 40 (1992) 1922–1924.

[4] Ehlers D., Pfister M., Bartholomae S., Analysis of Tahiti vanilla by high-performance liquid chromatography, Z. Lebensm. Unters. For. 199 (1994) 38–42.

[5] Ramaroson-Raonizafinimanana B., Gaydou E.M., Bombarda I., Hydrocarbons from three vanilla bean species: *V. fragrans*, *V. madagascariensis*, and *V. tahitensis*, J. Agric. Food Chem. 45 (1997) 2542–2545.

Table IV.
Comparison of the concentrations (% dry matter) of aromatic compounds of vanilla beans grown in shade houses (averages of plots having given beans) and beans grown in traditional under wood cultivation (average of two analysis).

Treatment	4-Hydroxybenzoic acid		Vanillic acid		4-Hydroxybenzaldehyde		Vanillin	
	Content	CV (%)	Content	CV (%)	Content	CV (%)	Content	CV (%)
T2	0.052	0,38	0.13	38,0	0.24	4,2	3.12	22,4
T3	0.050	12,00	0.18	11,1	0.20	15,0	3.28	7,3
T4	0.060	26,70	0.18	27,8	0.22	9,1	4.02	22,6
T5	0.044	25,00	0.17	35,3	0.24	20,8	3.76	9,3
T6	0.052	28,80	0.20	25,0	0.18	27,8	3.59	12,2
T7	0.059	16,90	0.18	33,3	0.24	16,7	3.97	32,0
T8	0.054	22,20	0.22	13,6	0.19	21,1	3.88	8,2
Under wood vanilla	0.046	–	0.072	–	0.23	–	2.2.	–

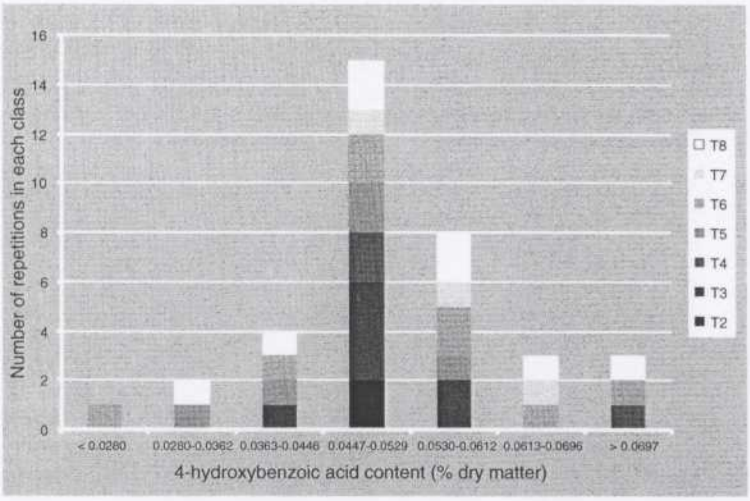


Figure 5.
Distribution of the repetitions of eight treatments testing the use of different soil amendments for vanilla cultivation, according to classes of bean of 4-hydroxybenzoic acid content. The treatments T1 to T8 are those presented in *table I*.

[6] Renaud G.S., Martin Y.L., Martin G.G., Martin G.J., Detection of sophisticated adulterations of natural vanilla flavors and extracts: application of the SNIF-NMR method to vanillin and p-hydroxybenzaldehyde, J. Agric. Food Chem. 45 (1997) 859–866.

[7] Bréas O., Fourel F., Martin G.J., ^{13}C analysis of aromas and perfumes by a coupled C-IRMS technique. The case of vanillin and leaf alcohol extracts, Analusis, 22 (1994) 268–272.

[8] Kaunzinger A., Juchelka D., Mosandl A., Progress in the authenticity assessment of vanilla. 1. Initiation of authenticity profiles, J. Agric. Food Chem. 45 (1997) 1752–1757.

[9] Cibes H.R., Childers N.F., Loustalot A.J., Influence of mineral deficiencies on growth and composition of vanilla vines, Plant Physiol. 22 (1947) 291–299.

[10] Bourriquet G., Les engrais chimiques et le vanillier, Agron. Tropic. 3 (1948) 32.

[11] Josselin C., Une des grandes richesses de Madagascar, Entreprises Produits Madagascar 9 (1951) 9–36.

[12] Gohier J.M., La culture du vanillier, Orchidophile 11 (1979) 1121–1128.

[13] Demarne F.E., Filière plantes aromatiques, In: Rapport annuel Cirad-Réunion, Cirad, Saint-Pierre, 1995.

[14] Odoux É., Filière plantes aromatiques, In: Rapport annuel Cirad-Réunion, Cirad, Saint-Pierre, 1996.

[15] Brunerie P.M., Procédé d'obtention d'arôme naturel de vanille par traitement enzymatique des gousses de vanille verte et arôme obtenu, International Patent Application, N PCT/FR 92/00837, 1991.

[16] Brodelius P.E., Phenylpropanoid metabolism in *Vanilla planifolia* Andr. (V) high performance liquid chromatographic analysis of phenolic glycosides and aglycones in developing fruits, Phytochem. Analysis 5 (1994) 27–31.

Efecto de las enmiendas en los principales compuestos aromáticos de la vaina de vainilla.

Resumen — Introducción. Dado el contexto económico de la isla de la Reunión y la falta de información fiable acerca de la fertilización de la vainilla, se efectuó un estudio para comparar el efecto, en el crecimiento de bejucos y los rendimientos de vainas, de diferentes tipos de enmiendas disponibles en la isla. Se evidenciaron diferencias muy significativas entre los tratamientos que afectan al crecimiento de bejucos y la inducción floral. El objetivo de dicho trabajo era estudiar el efecto de la composición de diferentes sustratos de cultivo en los principales compuestos aromáticos de las vainas. **Material y métodos**. Se probaron ocho enmiendas para la producción de vainilla (*Vanilla fragans*) en cultivo hidropónico sombreado. Las vainas se recolectaron en la fase de cosecha (color amarillo). Se emplearon como referencias vainas de vainilla cultivadas de forma tradicional. Tras extracción, cuatro compuestos aromáticos (vanilina, ácido vanillico, ácido 4-hidroxibenzoico y 4-hidroxibenzaldehido) se analizaron mediante HPLC. **Resultados y discusión**. Los contenidos aromáticos de vainas de los ocho tipos de enmiendas empleadas no presentaron diferencias significativas. Sin embargo, las vainas de vainilla cultivadas de forma tradicional en sotobosque parecen presentar diferencias cualitativas de contenido en vanilina y ácidos vanillicos con respecto a los valores de las parcelas experimentales bajo sombra. **Conclusión**. Habrá que realizar un experimento para identificar los parámetros responsables de las diferencias observadas entre estos dos tipos de cultivo de vainilla. © Éditions scientifiques et médicales Elsevier SAS

Vainilla / enmiendas organicas / compuestos aromáticos / Francia (Réunion)