Ripening of bananas using *Bowdichia virgilioides* Kunth leaves

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Bananas are usually ripened with calcium carbide (CaC₂), a dangerous substance that can cause food poisoning. The objective was to test the empirical ripening banana method using *Bowdichia virgilioides* leaves compared to carbide. Ripening tests were carried out using ‘Pacovan’ banana fruits with *B. virgilioides* leaves and carbide following the empirical method used by Borborema farmers, Paraíba, Brazil. *Bowdichia virgilioides* leaves induced increased respiration and ascorbic acid production and reduced acidity, chlorophyll and pH in banana fruits like CaC₂. Leaves of *B. virgilioides* induce ripening of ‘Pacovan’ banana with safer and same results than with CaC₂.

*Musa* sp. has global economic importance as one of the most important basic food sources along with rice, maize and wheat¹. About 150 countries produce this fruit, totaling more than 100,000 t year⁻¹. India (25,000 t year⁻¹), China (10,000 t year⁻¹), Philippines (8,900 t year⁻¹), Ecuador (6,770 t year⁻¹) and Brazil are the main banana producers in the world²⁻⁴. These countries have socioeconomic similarities highlighting the importance of this culture for the economy and regional development⁵, mainly for small producers⁶. Simplified cultivation processes, high demand and acceptance of bananas in the domestic market of these countries, enable their production, albeit often with low quality and/or productivity⁷. In addition, cooperatives and associations are important channels to organize and support banana farming activities⁸.

Banana fruit ripening depends on intrinsic factors such as respiration and ethylene production/sensitivity⁹ and market requirements¹⁰. Locally marketed bananas may be harvested at a later maturation stages, but bananas for export should be harvested the day before or the day of shipment¹¹. In this case, maturation standardization is induced by air conditioning¹² to plan banana commercialization and industrialization¹³. Acetylene and traces of this compound, produced by calcium carbide (CaC₂), accelerate and standardize ripening (color uniformity) without losses to quality or taste¹⁴. These products cannot be used in organic or agroecological production systems¹⁵, but have no restrictions in countries such as Bangladesh, India, Nepal and Pakistan¹⁶.

CaC₂ can cause adverse effects to human health, such as choking, motor coordination problems, headaches, respiratory tract inflammation, respiratory system irritation, mucous membrane and skin burns and reduction of the oxygen supply to the brain due to the chemical reaction of this product with water¹¹. Effective, low-cost and natural methods can avoid the harmful health effects of ripening chemical inducers.

Merchant producers of the Borborema polo, Paraíba state, Brazil mature bananas with *Bowdichia virgilioides* Kunth (Fabaceae) leaves with results like those obtained with CaC₂ but at lower cost. The ripening process with leaves of this plant includes the collection of *B. virgilioides* leaves at the coolest time of day, avoiding dew and excessive humidity during subsequent stages. The leaves of this plant are placed on the ground and the banana fruits are placed over them, but the proportion of leaves and fruits is not precise. The bananas and the leaves are
then covered with plastic sheeting without air exchange between the external and internal environments. The tarp is left for 24 hours or longer depending on the fruit quantity. Masonry tanks of 1 m² are also used with this method (personal farmer communication, 2017).

The sustainable management of *B. virgilioides* plants can facilitate the use of its leaves to induce banana ripening more economically. The objective was to test the empirical method of banana maturation using *B. virgilioides* leaves compared to the conventional method with CaC₂.

### Results

Ethylene concentration was higher in the atmosphere of the treatments with 4, 6, 8, and 10 g of leaves *B. virguloides*, followed by treatment with CaC₂ in the laboratory ripening trial. In the field ripening trial, the ethylene concentration was higher in atmosphere of the treatment with leaves *B. virguloides*, followed by treatment with CaC₂. Acetylene (C₂H₂) was only detected on treatment with CaC₂ in the laboratory and field ripening trial. Respiratory rate, ascorbic acid content, malic acid, pH, and chlorophyll in 'Pacovan' bananas matured with 2, 4, 6, 8 e 10 g of *Bowdichia virgilioides* leaves (T1, T2, T3, T4 and T5), carbide (g 50 Kg⁻¹ carbide) (T6) and, uncoated with plastic film (T7) fruits covered with plastic film (T8) in the laboratory ripening trial and LBr = *B. virgilioides* leaves and CaC₂ = calcium carbide in the field laboratory ripening trial. nd = not detected. *Means followed by the same letter per column do not differ by Tukey Test at 5% probability.

![Table 1](https://example.com/table1.png)

| Treat. | C₂H₄ | C₂H₂ | RR | AsA | MA | pH | Chlo |
|--------|------|------|----|-----|----|----|-----|
| T1     | 27.9 ± 2.1c | nd | 17.6 ± 1.6b | 16.1 ± 2.1b | 45.0 ± 2.5a | 5.6 ± 0.2a | 10.3 ± 0.7a |
| T2     | 66.7 ± 4.9a | nd | 48.6 ± 2.1a | 35.3 ± 3.6a | 38.8 ± 2.8a | 4.9 ± 0.2b | 10.8 ± 0.9a |
| T3     | 69.7 ± 5.8a | nd | 52.4 ± 3.0a | 40.9 ± 3.0a | 33.7 ± 2.1b | 5.0 ± 0.3b | 12.1 ± 0.1b |
| T4     | 68.4 ± 6.4a | nd | 54.6 ± 1.9a | 33.8 ± 2.9a | 32.6 ± 3.0b | 4.6 ± 0.5b | 12.1 ± 0.1b |
| T5     | 71.9 ± 7.4a | nd | 59.7 ± 3.1a | 42.6 ± 3.1a | 35.3 ± 3.0b | 4.5 ± 1.0b | 10.1 ± 0.1b |
| T6     | 32.7 ± 3.5b | nd | 387.2 ± 12.2 | 45.3 ± 3.6a | 45.7 ± 2.6a | 38.0 ± 2.9b | 4.5 ± 1.0b | 2.3 ± 0.1b |
| T7     | —     | —   | 18.3 ± 1.0b | 12.9 ± 0.9b | 42.1 ± 3.0a | 5.5 ± 1.1a | 11.1 ± 0.1a |
| T8     | 19.8 ± 1.4d | nd | 17.4 ± 0.9b | 13.2 ± 0.6b | 42.3 ± 2.4a | 5.9 ± 0.1a | 10.5 ± 0.1a |
| CV (%) | 11.78 | 10.1 | 13.12 | 17.98 | 8.43 | 4.37 | 3.14 |

### Discussion

The highest ethylene concentrations in the treatments with 4, 6, 8, and 10 g of *B. virgilioides* leaves are due to a cumulative effect of the gas produced by fruits and leaves. Ethylene concentration increases under atmospheric conditions modified by gas exchange limitation and autocatalysis17. The ethylene detected in the treatment with CaC₂ was the autocatalytic produced by the banana fruits and induced by the calcium carbide. CaC₂ may increase respiration rate, ethylene autocatalytic, chlorophyll degradation, carotenoid synthesis, starch conversion to sugar, increased activity of cell wall enzymes degradation, color change, texture, fruit aroma, and taste16. Acetylene is an ethylene analog used to initiate fruit ripening16. However, acetylene has lower biological activity than ethylene and higher concentrations for the same exposure period and for the same responses are needed16. In bananas, 0.01 ml L⁻¹ of ethylene at 18 °C for 24 h began to ripen, while 1.0 ml L⁻¹ of acetylene was required for a similar effect in several Florida hose cultivars19. The C₂H₂ was only detected on treatment with CaC₂ due to the presence of this compound which is industrially produced and only releases C₂H₂ when reacted with water21.

The increase in the respiratory rate of 'Pacovan' bananas ripened with 6, 8, and 10 g of *B. virgilioides* leaves and CaC₂ is due to the climacteric induction of respiration by the ethylene and acetylene emanated by *B. virgilioides* leaves and CaC₂, respectively. Phosphofructokinase activity, which regulates this pathway13, produces energy (ATP) from starch degradation and hexose oxidation resulting in climacteric respiration22. In addition, the fruit exposure to ethylene and acetylene produced by *B. virgilioides* leaves and CaC₂, respectively, may have increased the activity of the enzymes synthase and oxidase of ACC23 inducing climacteric respiration and accelerating maturation.
The highest ascorbic acid content (AsA) in bananas ripened with 6, 8, and 10 g of *B. virgilioides* leaves and CaC₂ is due to the higher demand for AsA in these fruits, presumably by the most oxidized redox cell state. The early fruit ripen, induced by ethylene and acetylene, produces reactive oxygen species increasing the demand for AsA reacting with superoxide, hydroxyl and peroxyl radicals, hydrogen peroxide, hypochlorite and singlet oxygen. However, the biosynthesis of AsA is an antioxidant response by the D-glucosone, D-galacturonate, myo-inositol and D-mannose/L-galactose pathways but not necessarily related to its accumulation.

The reduction of ripen 'Pacovan' banana acidity (malic acid) with 6, 8, and 10 g of *B. virgilioides* leaves and CaC₂ is due to the oxidation of organic acids during fruit ripening by the increase in tricarboxylic acid cycle activity. These acids were more rapidly and extensively degraded during the climacteric respiration induced by ethylene and acetylene, emanating from the *B. virgilioides* leaves and CaC₂, respectively.

The pH reduction in 'Pacovan' bananas can be explained by the increase in the ascorbic acid content exceeding the titratable acidity reduction in the fruits matured with 6, 8, and 10 g of *B. virgilioides* leaves and CaC₂. The AsA accumulation reduced the pH of these fruits due to the acidic character of this molecule attributed to the enodiol group. The hydrogens of the enodiol group can dissociate, resulting in the strong ascorbic acid acidity and therefore are potential reducing agents.

The lowest concentration of total chlorophyll in the 'Pacovan' banana peel ripe fruit induced with 6, 8 and 10 g of *B. virgilioides* leaves is due to the structural decomposition of chlorophyll by chlorophyllases, stimulated by ethylene and acetylene emanated from leaves and CaC₂, respectively. The increase in the activity of these enzymes in these treatments coincides with the climacteric increase in fruit respiration, which was also induced by ethylene and acetylene. Ethylene and acetylene its analogues accelerate the chlorophyll losses and regulates the yellowing of banana peels. The drop of total chlorophyll concentration in banana fruits induced by CaC₂ was similar due lower biological activity than ethylene and higher concentrations for the same exposure period and for the same responses are needed.

**Conclusion**

The method used by Borborema producers in the Paraíba state, Brazil to ripen 'Pacovan' bananas with *Bowdichia virgilioides* leaves is safer and has the same results than those obtained with carbide.
Material and Methods

Location and raw material. Banana bunches of the ‘Pacovan’ variety, from agroecological production, were harvested in the early hours in the morning. Banana fruits were selected and standardized according to size, absence of physiological defects and infections, at the maturation stage 3 with yellowish green color\(^5\). Part of the harvested fruits were transported to the laboratory and part remained in the field. Banana ripening was evaluated with \(B. \ virgilioides\) leaves (harvested according to the producers orientation) and calcium carbide (\(\text{CaC}_2\)) in the field and laboratory.

Laboratory ripening trial. ‘Pacovan’ banana bites were scrapped with a stainless-steel knife and the fruits were, individualized in trays of expanded polystyrene for 30 min, to reduce the ethylene effect produced in their wound. The treatments were 2.0 g of \(B. \ virgilioides\) leaves + plastic film coating (T1); 4.0 g of \(B. \ virgilioides\) leaves + plastic film coating (T2); 6.0 g of \(B. \ virgilioides\) leaves + plastic film coating (T3); 8.0 g of \(B. \ virgilioides\) leaves + plastic film coating (T4); 10.0 g of \(B. \ virgilioides\) leaves + plastic film coating (T5); \(\text{CaC}_2\) (g 50 kg\(^{-1}\)) + coating with plastic film (T6); internal control (only coated with plastic film) (T7) and external control (without coating with plastic film) (T8) per banana. Treatments were stored at 27 ± 2 °C and relative humidity of 87 ± 5%. The fruits remained under these conditions for 48 hours and were then evaluated. The experiment was developed in triplicate.

Field ripening trial (empirical method). Two and a half kilograms of \(B. \ virgilioides\) leaves were placed covering the entire soil and 100 kg of banana fruits are placed over them. The bananas and the leaves were then covered with tarp without air exchange between the external and internal environments. The tarp was left for 24 hours (personal farmer communication, 2017). The same procedure was performed by replacing the leaves with \(\text{CaC}_2\) (0.5 kg\(^{-1}\) of fruit). The control consisted only of the fruits covered by the tarp.

Ethylene and acetylene quantification. Ten air samples were withdrawn with syringes from the atmosphere beneath the tarp. The syringes needle tip were sealed with rubber and immediately taken to the laboratory where were injected into a GC-14B (ShimadzuCrop Kyoto Japan), with Porapak-Q packaged column and flame ionization detector for ethylene and acetylene analysis.

Respiratory rate. Banana fruits were placed in hermetically sealed containers with 10 mL of 0.5 N NaOH. The \(\text{CO}_2\) produced by the fruits, was measured by titration and after 24 h, the NaOH was titrated with 1 N HCl (without coating with plastic film) (T8) per banana. Treatments were stored at 27 ± 2 °C and relative humidity of 87 ± 5%. The fruits remained under these conditions for 48 hours and were then evaluated. The experiment was developed in triplicate.

Ascorbic acid. ascorbic acid content was determined by titration with a 0.02% 2,6-dichlorophenol-indophenol (DFI) indicator solution in 5.0 g of fresh banana mass diluted in 30 ml of oxalic acid at 0.5%\(^6\).

Titratable acidity. The malic acid content (acidity) of the banana was determined by titrometry in five-gram pulp samples of the fruit diluted in 50 mL of distilled water. Then, 4–5 phenolphthalein indicator droplets were added and titration performed with 0.1 N NaOH\(^4\). pH. The pH was determined in digital bench pH meter in samples, 30 min after the dilution of five grams of banana pulp in 50 ml of distilled water.

Total chlorophyll. Two grams of the banana peel were macerated with 7 mL of 80% (v/v) acetone and the extract filtered and the volume then filled with 80% (v/v) acetone. The absorbance was read at wavelengths at 646.8 and 663.2 nm, and calculated by the equation: total chlorophyll (T) = 7.15 × A\(_{663.2}\) + 18.71 × A\(_{646.8}\)\(^4\).

Experimental design and data analysis. The experiment was carried out in a completely randomized design with eight treatments and four replications. Each experimental unit had one banana per tray. The results were submitted to variance analysis by the F test and the means compared by the Tukey test (P < 0.05) with the program Assistat version 7.7.

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Acknowledgements
To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG) and “Programa Cooperativo sobre Proteção Florestal/PROTEF do Instituto de Pesquisas e Estudos Florestais/PEF” for financial support. Dr. Phillip John Villani (University of Melbourne, Australia) revised and corrected the English language used in this manuscript.

Author Contributions
W.S.R., R.C.N. and O.O.F designed the research. R.C.N., O.O.F and L.S.R. performed the experiments. W.S.R., E.L.P., J.C.Z., L.S.R. and M.B.A. wrote the manuscript. All authors approved the manuscript.

Additional Information
Competing Interests: The authors declare no competing interests.

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