Red Guava Leaf Harvesting Impact on Flavonoid Otimization in Different Growth Phases

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Received February 4, 2011/Accepted July 29, 2011

INTRODUCTION

Harvesting process is a critical time to identify the quality of raw material for traditional medicine. The time and harvesting techniques, drying process after harvesting, and processing to make the simplicia, are the crucial role to make the good quality of the natural product. The plant started the generative phase, were one of the factor that influenced the assimilate. Trentacoste et al. (1999), and has also been used extensively as a hypoglycaemic agent. Many pharmacological studies have demonstrated the ability of this plant to exhibit antioxidant, hepatoprotection, anti-allergy, antigenotoxic, antiplasmodial, cytotoxic, cardioactive, anticough (Garcia et al. 2003), antiadipetic, antiinflammatory activities, supporting its traditional uses, that suggested a wide range of clinical applications for the treatment of infantile rotaviral enteritis, diarrhoea and diabetes. Garcia et al. (2003) stated that other uses of guava leaves extract are as antimutagenic, and as cure for asthma. Cushnie and Lamb (2005) stated that increasing, this class of natural products is becoming the subject of anti-infective research, and many groups have isolated and identified the structures of flavonoids possessing antifungal, antiviral, and antibacterial activity. Moreover, several groups have demonstrated synergy between active flavonoids as well as between flavonoids and existing chemotherapeutics. The extraction of the phenolic fraction from guava seeds (Psidium guajava L.) at various operating conditions was explored (Castro-Vargas et al. 2010), but not from the leaves.

Leaf harvest will decrease the leaf number per plant, which also is one of pruning product. Dickson et al. (2000) found that different growth phases in plant, when the plant was dominated by vegetative growth or when the plant started the generative phase, were one of the factor that influenced the assimilate. Trentacoste et al. (2010) showed that on olive tree, the olive oil yield and its components (fruit number, average fruit weight, and fruit...
The research was carried out on March to November 2006 at Biopharmaca Research Station Bogor Agricultural University and Bogor Agro Lestari Laboratory cooperating with Post Harvest Research and Development Institute Bogor, Indonesia. Four-year-old red guava trees from air layering, hand counter, and UV spectrometer were used.

The red guava plants were planted in a terraced land with 30° incline. Pretreatment by picking all the flower and fruit were used to uniform the phases of each of the treatments and given at the same time (0 WO). Liming with 0.5 kg dolomit plant⁻¹ and 10 kg lamb manure plant⁻¹ treatments and given at the same time (0 WO). Liming fruit were used to uniform the phases of each of the growth phases and the amount of leaf harvested to the growth and development of red guava and flavonoid production from the leaves.

**MATERIALS AND METHODS**

The research was aimed to study the influence of plant protection, etc. (nutrient requirements, productivity at different scales but also for solving different management problems (nutrient requirements, plant protection, etc.). This research was aimed to study the influence of plant growth phases and the amount of leaf harvested to the growth and development of red guava and flavonoid production from the leaves. The red guava plants were handpicked from the tip to the base of the tertiary to pentanary branches, without the leaf bud. If there is no leaf on the tertiary branch, then the leaf from the tertiary lateral branches will be harvested. The second harvest was at the end of the experiment (22 WO). Rutin (molecules weight = 610.53 dalton) were used as standard for quantitative analysis of total flavonoid.

Variables observed from 2-22 WO were: the leaf number increase, tertiary and quartenary branches number, the time of quartenary branches appearance, fruits number, leaf wet and dried weight (at the beginning and the end of the experiment), leaf area index (LAI, at the beginning and the end of the experiment), leaf area plant⁻¹ (=leaf weight plant⁻¹/leaf weight 50 leaf⁻¹ x leaf area 50 leaf⁻¹; leaf area 50 leaf⁻¹ = length x width of the 50 leaf sample), flavonoid production (= flavonoid concentration in the leaf x leaf dry weight) at the beginning and the end of the experiment.

**RESULTS**

**Overall Condition.** Pretreatment to make the plants uniform were given in the middle of March 2006, with rain intensity 138.30 mm month⁻¹ and 84% humidity. The first leaf harvesting was at the end of May 2006, with high rain intensity of 324 mm month⁻¹. Average humidity was almost the same from the condition at the beginning of the uniforming the plants. Rain intensities fluctuated up to the end of the experiment (at beginning of November 2006), but for overall throughout the experiment, the months were dry months (Figure 1). Overall condition of the leaf, flower and fruit growth can be seen at Table 2.

Growth phases significantly affected the fruit number at 1-13 WO. Harvesting significantly affected the leaf number 1-17 WO, the tertiary branches number 10-22 WO, the quartenary branches number 6-22 WO, the time of quartenary branches appearance and leaf wet weight at the end of the experiment (Data not showed). Treatments interaction significantly affected flavonoid production at the end of the experiment.

**Leaf Increase.** The two growth phases had almost similar leaf increase, but at 2-10 WO, the vegetative phase plants treatment had more increase than generative phase; at 11-17 WO the generative phase plants had more leaf increase, and at 18-22 WO the vegetative phase plants had more leaf increase (Data not showed). Table 3 showed

<table>
<thead>
<tr>
<th>Table 1. Leaf harvesting treatment of red guava</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination of leaf harvesting treatment</td>
</tr>
<tr>
<td>Initial harvest of tertiary branches at 0 WO (the beginning of the observation)</td>
</tr>
<tr>
<td>Tertiary branch</td>
</tr>
<tr>
<td>% leaf harvested</td>
</tr>
<tr>
<td>0 (without)</td>
</tr>
<tr>
<td>25</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1  2  3  4   5  6   7  8   9 10 11 12 13 14 15 16 17 18 19 20 21 22</td>
<td></td>
</tr>
<tr>
<td>1.21 fruit/plant 7 WO</td>
<td>324 mm/month</td>
<td>229.03 leaf/plant 15 WO</td>
</tr>
<tr>
<td>46.68 g</td>
<td>191.2 mm/month</td>
<td>173 mm/month</td>
</tr>
<tr>
<td>17.33 g</td>
<td>152 mm/month</td>
<td>15.7 mm/month</td>
</tr>
<tr>
<td>173 mm/month</td>
<td>5.7 mm/month</td>
<td>25.7 mm/month</td>
</tr>
</tbody>
</table>

Figure 1. Leaf number increase, fruit number and flavonoid production at the beginning and the end of the experiment. Flavonoid production, Fruit number, Rainfall intensity.
Table 2. Overall condition of the leaf, flower, and fruit growth

<table>
<thead>
<tr>
<th>Harvesting</th>
<th>Sites of new leaf growth, flower and fruit growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tip of branches</td>
<td>- New lateral branches at the tip of the branches</td>
</tr>
<tr>
<td></td>
<td>- In the middle of the branches that has no leaf</td>
</tr>
<tr>
<td>100% tertiary branches with more than 6 pairs of leaf</td>
<td>- Quaternary branches formed</td>
</tr>
<tr>
<td>25% tertiary branches</td>
<td>- Restricted flowers growth or aborted</td>
</tr>
</tbody>
</table>

Table 3. Leaf increase affected by leaf harvesting

<table>
<thead>
<tr>
<th>Weeks after pretreatment</th>
<th>Leaf harvesting (%)</th>
<th>Leaf wet weight</th>
<th>Leaf dried weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>114.00a</td>
<td>41.25b</td>
<td>-41.25c</td>
</tr>
<tr>
<td>3</td>
<td>156.13a</td>
<td>81.00b</td>
<td>3.13b</td>
</tr>
<tr>
<td>4</td>
<td>185.00a</td>
<td>113.00ab</td>
<td>26.00c</td>
</tr>
<tr>
<td>5</td>
<td>155.63a</td>
<td>137.25a</td>
<td>4.38b</td>
</tr>
<tr>
<td>6</td>
<td>142.75a</td>
<td>165.50a</td>
<td>18.63b</td>
</tr>
<tr>
<td>7</td>
<td>103.38a</td>
<td>137.25a</td>
<td>4.38b</td>
</tr>
<tr>
<td>8</td>
<td>79.50ab</td>
<td>120.75a</td>
<td>11.13b</td>
</tr>
<tr>
<td>20</td>
<td>-48.30b</td>
<td>270.40a</td>
<td>158.40ab</td>
</tr>
<tr>
<td>21</td>
<td>-25.80b</td>
<td>312.80a</td>
<td>197.50ab</td>
</tr>
<tr>
<td>22</td>
<td>-3.50b</td>
<td>354.80a</td>
<td>227.10ab</td>
</tr>
</tbody>
</table>

Figures followed by different letters in the same row significantly different at 5% DMRT.

Table 4. The number of tertiary and quartenary branches

<table>
<thead>
<tr>
<th>Treatment</th>
<th>The number of tertiary branches.plant−1</th>
<th>The number of the first quartenary branches.plant−1</th>
<th>The time of quartenary branches appearances (WO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Phases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>14.4</td>
<td>17.38</td>
<td>1.07</td>
</tr>
<tr>
<td>Generative</td>
<td>9.88</td>
<td>5.00</td>
<td>0.94</td>
</tr>
<tr>
<td>Leaf Harvesting (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.63</td>
<td>0.75</td>
<td>11.50b</td>
</tr>
<tr>
<td>25</td>
<td>1.13</td>
<td>1.25</td>
<td>31.25a</td>
</tr>
<tr>
<td>50</td>
<td>0.63</td>
<td>1.00</td>
<td>28.00a</td>
</tr>
<tr>
<td>100</td>
<td>1.25</td>
<td>1.13</td>
<td>12.88b</td>
</tr>
</tbody>
</table>

Figures followed by different letters in the same row significantly different at 1% DMRT test, log x for leaf number and (x+1.5)\(^{1/2}\) for the number of tertiary and quartenary branches.

Table 5. Wet and dried leaf weight at the beginning and the end of the experiment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>The beginning of the experiment</th>
<th>The end of the experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf wet weight</td>
<td>Leaf dried weight</td>
</tr>
<tr>
<td>Growth Phase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>200.19b</td>
<td>93.89b</td>
</tr>
<tr>
<td>Generative</td>
<td>343.31a</td>
<td>157.92a</td>
</tr>
<tr>
<td>Leaf Harvesting (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>277.96</td>
<td>135.90</td>
</tr>
<tr>
<td>25</td>
<td>179.60</td>
<td>83.36</td>
</tr>
<tr>
<td>50</td>
<td>297.45</td>
<td>131.61</td>
</tr>
<tr>
<td>100</td>
<td>332.00</td>
<td>152.74</td>
</tr>
</tbody>
</table>

Figures followed by different letters in the same column significantly different at 1% DMRT test, with (x+1.5)\(^{1/2}\) transformation.
on 50% leaf harvesting in generative phase (89.90 g), which is 1149% higher.

**Interaction of Growth Phase and Leaf Flavonoid Production with Time.** Growth phase, leaf harvesting and

### Table 6. Leaf area index

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LAI of the experiment</th>
<th>at the beginning</th>
<th>at the end</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth Phases</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetative</td>
<td>0.46</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>Generative</td>
<td>0.43</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td><strong>Leaf Harvesting (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.59</td>
<td>0.27ab</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.41</td>
<td>0.47a</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.42</td>
<td>0.42b</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.37</td>
<td>0.12b</td>
<td></td>
</tr>
</tbody>
</table>

Figures followed by different letters in the same column significantly different at 1% DMRT test, with $(x+1.5)^{1/2}$ transformation.

**DISCUSSION**

Guédon *et al.* (2007) stated that observed growth, as given, for instance, by the length of successive annual shoots along the main axis of a plant, is mainly the result of two components: an ontogenetic component and an environmental component. In this experiment, LAI used as one of relative growth rate (RGR) variable based on leaf weight, whereas Sulistijorni *et al.* (2008) used plant height. The result of the harvesting treatment showed that no leaf harvesting and 100% leaf harvesting significantly had the lowest leaf flavonoid production than 50% leaf harvesting, whereas 25% leaf harvesting had the same leaf flavonoid production with the other three treatments. Trees with no leaf harvesting treatment experience no disturbance because of the leaf harvesting at the beginning of observation, such as that the tree grow at the normal growth through the experiment. The flower and fruit still intact on trees on generative phase gave the needed sink, caused the partitioning of the photosynthate for the growth and the generative organ, and made the leaf flavonoid production lower (16.87g tree$^{-1}$) than on vegetative phase (32.53 g tree$^{-1}$).

No leaf harvesting treatment had the highest leaf increase at 2 to 4 WO, the highest leaf abscission l at 20 WO (November 2006), the slowest quartenary branches (5.5 WO) emergence, LAI at the beginning (0.59) = 2 at the end of the experiment (0.27), the lowest number of tertiary (11.13) and quartenary branches at 22 WO (11.50). This treatment experience no induction of pruning effect, whereas on vegetative phase, the flower and fruit picking

### Table 7. Treatments interaction on leaf flavonoid production at the end of the experiment

<table>
<thead>
<tr>
<th>Growth phases</th>
<th>Harvesting (%)</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetative</td>
<td>0.53ab 69.06ab</td>
<td>44.77A</td>
</tr>
<tr>
<td>Generative</td>
<td>35.45ab 89.90a</td>
<td>48.58B</td>
</tr>
<tr>
<td>Average</td>
<td>32.70B 79.77A</td>
<td>29.96B</td>
</tr>
</tbody>
</table>

Figures followed by different small letters in the same rows or column significantly different at 1% DMRT test, with $(x+1.5)^{1/2}$ transformation.

### Table 8. Leaf production at the beginning and the end of the experiment

<table>
<thead>
<tr>
<th>Time of observation</th>
<th>Leaf wet weight·plant$^{-1}$</th>
<th>Leaf dried weight·plant$^{-1}$</th>
<th>LAI</th>
<th>Flavonoid production·plant$^{-1}$</th>
<th>Leaf flavonoid concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning</td>
<td>271.75</td>
<td>132.28</td>
<td>0.45</td>
<td>17.33b</td>
<td>138.08b</td>
</tr>
<tr>
<td>End</td>
<td>194.48</td>
<td>109.98</td>
<td>0.32</td>
<td>46.68a</td>
<td>437.94a</td>
</tr>
</tbody>
</table>

Figures followed by different small letters in the same column significantly different at 1% DMRT test, with $(x+1.5)^{1/2}$ transformation.
had negative effect on the trees, and caused the trees to have no sink and the highest leaf abscission.

Trees with 100% leaf harvesting treatment had the most severe treatment from leaf harvesting and forced condition of vegetative phase treatment, so that made the trees had the lowest flavonoid production (7.82 g tree⁻¹) than all the other treatments. Moderate leaf number increase, the lowest LAI at the beginning (0.37) and the end (12.88) of the experiment, leaf abscission occurred especially on vegetative phase, with smaller leaf size and the lowest leaf water content in the end of the experiment (20.53%). High stress caused the trees with 100% leaf harvesting on vegetative phase produced smaller leaf size in lower quantity on the lowest number of leaf on tertiary branches (21.88) and quartenary branches (12.88) at 22 WO.

Trees with 25% leaf harvesting treatment had more leaf increase on vegetative phase than generative phase, that made the similar trend in leaf flavonoid production. The effect of pruning was the second highest on this treatment, causing the highest number of leaf increase (354.8 tree⁻¹), the highest number of tertiary (20.63 tree⁻¹) and quartenary branches (31.25 tree⁻¹), the lowest wet weight at the beginning (179.60 g tree⁻¹), but the highest wet weight at the end of the experiment (301.31 g tree⁻¹), leaf water content 47.91% at 22 WO, and almost the same LAI value (0.41 at the beginning, and 0.47 at the end), almost the same number of leaves found on tertiary and quartenary branches. The data suggested that 25% leaf harvesting showed the stabilized growth of the guava.

Trees with 50% leaf harvesting treatment had the most severe negative effect on leaf number increase (41.25 tree⁻¹) from first harvest that caused leaf abscission, but leaf number increase was moderate at 22 WO which was similar with 100% leaf harvesting treatment. The first harvest also caused the lowest number of tertiary branches (0.63 tree⁻¹) at 1 WO and lowest tertiary branches (12.88 tree⁻¹) at 22 WO, but the highest quartenary branches (28 tree⁻¹) at 22 WO, the same LAI value (0.42), and the second highest wet weight at the beginning and the end of the experiment. Treatment of 50% leaf harvesting promoted more quartenary branches with most of leaves on these branches, this data also suggested a stabilized growth. Treatment of 50% leaf harvesting had the similar highest leaf number increase at 15 WO/September and 22 WO/November (414.75 g tree⁻¹).

From 11 WO of the experiment, the tree with vegetative phase treatment almost had no fruit left to the end of the experiment. Whereas the generative still had 1 fruit tree⁻¹ up to 10 WO, and declining to the end of the experiment. Vegetative phase had lower LAI (99.82 tree⁻¹), and higher water leaf content (51.32%) than generative phase (115.45 tree⁻¹ and 43.45%). The data suggested that leaves on generative phase weight more than on vegetative phase. On the other hand flavonoid content of the leaf was also higher at the end (437.94 ppm) than at the beginning of the experiment (138.08 ppm).

Maintenance respiration costs, which are calculated on the basis of the $Q_{10}$ concept, have first priority. Vegetative and reproductive growth are given second and third priority. Daily carbon demands for the vegetative and reproductive organs are based on an analytical formulation of the potential growth rate at any time (Lescourret et al. 1998).

Fruit number on 50% leaf harvest (0.75 plant⁻¹) was significantly higher than the other leaf harvest treatments at 20 WO, was playing an important role for the sink. Lescourret et al. (1998) also stated that in a simulation model of daily C assimilation and allocation in an isolated shoot-bearing fruit, the pool of C assimilates available daily for distribution is the daily assimilation of C, plus that mobilized from reserves if the demand of sink organs exceeds the product of photosynthesis. Lopez et al. (2008). Carbohydrate partitioning was related to carbon allocation, such as organ growth, carbohydrate assimilation, reserve dynamics, and respiration maintenance.

Rain intensities fluctuated up to the end of the experiment (at beginning of November 2006), but for overall throughout the experiment, the months were dry months. Drought has dramatic negative effects on plants’ growth and crop productivity. Although some of the responses and underlying mechanisms are still poorly understood, there is increasing evidence that drought may have a negative effect on photosynthetic capacity (Damour et al. 2008). This condition made the red guava trees forced on the generative state, not on flush state. Chaubert-Pereira et al. (2010), stated that tree growth is assumed to be mainly the result of three components: (i) an endogenous component assumed to be structured as a succession of roughly stationary phases separated by marked change points that are asynchronous between individuals; (ii) a time-varying environmental component assumed to take the form of synchronous fluctuations between individuals; (iii) an individual component corresponding mainly to the local environment of each tree. The result suggested that harvesting 50% leaf on tertiary branches in generative phase can be used to produce the highest flavonoid (89.90 g tree⁻¹) on red guava leaf.

REFERENCES


