Differences in Floral Morphology, Floral Nectar Constituents, Carotenoids, and Flavonoids in Petals of Orange and Yellow *Pyrostegia venusta* (Bignoniaceae) Flowers

By

Antônio Barioni GUSMAN*) and Gerhard GOTTSSBERGER**)

With 2 Figures

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Summary


Orange-colored flowers of *Pyrostegia venusta* (Bignoniaceae) from the Brazilian cerrado vegetation were analyzed and compared with yellow-colored ones with regard to morphological parameters, nectar constituents, and petal pigments. In
comparison to the yellow flowers, the more common orange ones are characterized by a wider floral tube, a higher total nectar production, a higher sucrose content, a higher amino acid content per volume, and a higher concentration of carotenoids in the petals. On the other hand, flavonoids are more concentrated in the yellow flowers. Legitimate floral visitation by hummingbirds and nectar robbing by bees are discussed. However, a direct comparison of the flower visitors was not possible, since the whole mixed population was destroyed by agricultural activity.

Zusammenfassung


Introduction

Pyrostegia venusta (Ker-Gawl.) Miers, a neotropical heliophyte bignoniaceous vine is a typical colonizing species. It is usually found in secondary growth of the Atlantic forests, and in “Cerrado”, a characteristic savanna-like vegetation. Its geographical distribution reaches from southern Brazil to Paraguay, Bolivia, and northeastern Argentina (SANDWITH & HUNT 1974).

Normally, this species displays orange flowers; however, we have noticed some individuals with yellow flowers in a population in an altered part of a cerrado area in the state of São Paulo.

Flowering of orange- and yellow-flowered individuals of P. venusta was synchronized, and lasted for 3 months. Following seed ripening the aerial parts dry up, or may be destroyed by fire.

The basic morphology of the flowers was described by GENTRY 1974, who included Pyrostegia in the Martinella type. Pyrostegia flowers fit the syndrome of hummingbird pollination (FAEGRI & VAN DER PIJL 1971, RAVEN 1972, PROCTOR & YEO 1973). Studies in floral biology and nectar secretion of orange flowers of this species were conducted by GOBATTO-RODRIGUES & STORT 1992 and GALETTO & al. 1994. Further information is more cursory
and refers to the activity of legitimate and illegitimate flower visitors and to the variation of amino acids and sugars in floral nectar (GENTRY 1978, CAMARGO & al. 1984, GOTTBERGER & al. 1984).

In this paper we describe differences between the two floral color forms with respect to floral morphology, amount of floral nectar and its sugar content, composition of nectar amino acids, amount of total carotenoids, and presence of flavonoids in petals. These parameters in relation to the known behavior of flower visitors form the base for our speculation about the significance of the differently colored floral forms for the reproductive biology of *P. venusta*.

**Material and Methods**

Field work was conducted in the municipality of Santa Rita do Passa Quatro, northeastern São Paulo State, Southeastern Brazil, during August and September 1982, within an area of about 5 ha of typical cerrado vegetation, named “Cerrado de Agua Espalhada” (altitude 780 m, 21° 45' S, 47° 28' W). The whole population of *Pyrostegia venusta* occupied a disturbed patch of about 1.5 ha within this cerrado area. Individuals with orange flowers outnumbered those with yellow flowers by about 2 to 1.

Whole inflorescences were collected and kept in styrofoam boxes until measurements of the morphological characters were performed. For each of the color forms thirty open flowers were chosen at random from several inflorescences in the population. Diameter of corolla tube entrance and corolla tube constriction, length and width of the nectary and length of the ovary were measured exactly and the means were calculated.

For nectar collection, whole inflorescences were enclosed in fine meshed nylon bags at about 18.00 p.m. The next morning, at about 9:00 a.m., only the freshly opened flowers were bagged again. After nectar accumulation over 10 hours, nectar (from 30 orange and 30 yellow flowers) was collected very carefully from the flowers with different micropipettes adapted to a rubber bulb. In order to avoid contamination with pollen when extracting nectar, the external parts of the corolla tubes with the stamens were gently cut off with scissors. In another experiment (using 20 orange and 20 yellow flowers) nectar was extracted from flowers twice during the period of 10 hours, once after 2 hours and a second time after 10 hours. Total daily nectar accumulation was calculated.

The samples were placed into small flasks of known weight and kept frozen until reweighing on a Mettler balance; the value obtained was converted to ml. The mixture of nectar extracted from 30 orange and 30 yellow flowers was kept in an ice box until analysis of amino acids and sugars.

Sugar concentration of the accumulated nectar was measured in sucrose equivalents with a hand pocket refractometer (Carl Zeiss). The reductive monosaccharides glucose and fructose and the non-reductive sucrose were determined according to DUBOIS & al. 1956. The amino acid content was determined directly from the nectar samples using an amino acid autoanalyzer Nicholas VI.

Total carotenoids were extracted and determined from weighted samples of orange and yellow corollas of *Pyrostegia* flowers according to BOOTH 1957. Flavonoids
were removed from the corollas by soaking them in methanol for one week (THOMPSON & al. 1972). The extracts were diluted and the relative absorption within a wavelength range of 220–500 mm was measured with a Perkin-Elmer spectrophotometer.

Results

Floral parameters: Orange- and yellow-flowered Pyrostegia individuals occurred in the same area and were blooming simultaneously and synchronically. Although at a glance the two flower morphs appeared to be very similar morphologically, the measurement revealed differences in corolla tube constriction, nectary width/length ratio, ovary length, and pollen/ovule ratio between orange and yellow flowers (Fig. 1, Tab. 1). Floral tube entrance diameters, corolla tube constriction, nectary width/length ratio, and pollen/ovule ratio were larger in orange than in yellow flowers. Only the ovary length is larger in the yellow flowers. These differences were significant at a 0.05 level (t-test).

Nectar, sugar production and amino acid data are presented in Tables 2 and 3. The nectar content after 10 hours of yellow flowers was significantly lower (48.6 ± 7.3 μl) than the nectar content of orange ones (56.8 ± 8.3 μl). The sugar content was 19% and 24% sucrose equivalent, respectively. These values were converted to mg sucrose per ml nectar (see C.R.C. Handbook of chemistry and physics 1975–76). These sugar concentrations by calculation provide about 806 cal and 1040 cal per ml of nectar, respectively. Following BOLTEN & al. 1979 and SOUTHWICK & al. 1981, the energetic reward was calculated for individual flowers. Total nectar production after double extraction reached even daily averages of 79.3 ± 16 μl and 101 ± 22 μl per flower, in yellow and orange flowers respectively. This total daily production by yellow and orange flowers can provide 267 J (= 64 cal) and 435 J (= 104 cal) flower/day, respectively, if we consider the concentration of sugar as sucrose equivalent.

Amino acids are present in nectar of yellow and orange flowers at concentrations of 7.47 μg/ml and 13.99 μl/ml nectar (Tab. 3). Yellow flowers showed a predominance of aspartic acid, glutamic acid, and alanine, while orange flowers are dominated by aspartic acid, alanine, and glycine, in decreasing order.

Carotenoids and flavonoid pigments: There were differences in carotenoid content (Tab. 2) and in the presence of flavonoids (Fig. 2) for orange and yellow corollas of Pyrostegia. Orange flowers are much richer in total carotenoids than yellow ones, but yellow flowers have a higher flavonoid concentration than orange ones. While the common orange colored flower looks evenly orange at first glance, a closer examination reveals that small inner portions at the base and the middle of the corolla are, in fact, yellowish.
Fig. 1. a. Yellow (above) and orange (below) color forms, with flower tube differences slightly exaggerated. – b. Corolla tube with tube entrance (e) and corolla tube constriction (c). – c. Longitudinal section through the basal part of an orange-colored flower, with calyx, corolla tube basis, and ovary. – d. Section through a yellow-colored flower. – l = length, w = width of nectary, o = ovary, p = petal, s = sepal.

A comparison of the absorption spectra of yellow and orange corolla extracts (Fig. 2) shows that both have the carotenoid absorptions between 430 and 470 mm. In the ultraviolet part of the spectrum yellow flowers had a higher absorption than orange ones (maxima at 330 mm with shoulders at 295 mm).
### Table 1
Means and standard deviations of floral parameters for orange and yellow flowers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Orange (n=30)</th>
<th>Yellow (n=30)</th>
<th>Value of ta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter of corolla tube entrance (cm)</td>
<td>1.35 ±0.071</td>
<td>0.98 ±0.070</td>
<td>20.32</td>
</tr>
<tr>
<td>Diameter of corolla tube constriction (cm)</td>
<td>0.33 ±0.14</td>
<td>0.24 ±0.090</td>
<td>2.96</td>
</tr>
<tr>
<td>Nectary length/width ratio (cm)</td>
<td>1.10 ±0.032</td>
<td>0.74 ±0.056</td>
<td>30.57</td>
</tr>
<tr>
<td>Ovary length (cm)</td>
<td>0.47 ±0.016</td>
<td>0.53 ±0.02</td>
<td>10.77</td>
</tr>
<tr>
<td>Pollen/ovule ratio</td>
<td>312 ±45</td>
<td>205 ±19.6</td>
<td>11.96</td>
</tr>
</tbody>
</table>

a = t tests; = 0.05

### Table 2
Total nectar production after 10 h accumulation, sugar and carotenoid concentrations of orange and yellow flowers

<table>
<thead>
<tr>
<th></th>
<th>Nectar accumulation µl/flower</th>
<th>Reductive sugars mg/ml</th>
<th>Sucrose concentration mg/ml</th>
<th>Carotenoids mg/g/petal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Orange (n=30)</strong></td>
<td>56.8 ±8.30</td>
<td>56.6 ±4.81</td>
<td>263.8</td>
<td>1.91 ±0.05</td>
</tr>
<tr>
<td><strong>Yellow (n=30)</strong></td>
<td>48.6 ±7.30</td>
<td>67.1 ±5.20</td>
<td>204.5</td>
<td>0.87 ±0.08</td>
</tr>
</tbody>
</table>

### Table 3
Amino acid constituents in nectar of orange and yellow flowers (µg/ml)

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Orange (n=30)</th>
<th>Yellow (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>2.67</td>
<td>0.90</td>
</tr>
<tr>
<td>Arg</td>
<td>0.21</td>
<td>0.42</td>
</tr>
<tr>
<td>Asp</td>
<td>8.19</td>
<td>4.00</td>
</tr>
<tr>
<td>Glu</td>
<td>0.83</td>
<td>0.59</td>
</tr>
<tr>
<td>Gly</td>
<td>1.50</td>
<td>1.13</td>
</tr>
<tr>
<td>Lys</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Ser</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Val</td>
<td>0.18</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>13.99</td>
<td>7.47</td>
</tr>
</tbody>
</table>
Hummingbirds are the regular pollinators of *Pyrostegia venusta* flowers. GOBATTO-RODRIGUES & STORT 1992 found three hummingbird species, Eupetomena macroura, Amazilia lactea, and Phaethornis pretrei, visiting flowers in southern Brazil, and GALETTO & al. 1994 Chlorostilbon aureoventris and Sappho sparganura around Cordoba, Argentina. GENTRY 1974 and CAMARGO & al. 1984 also mention hummingbirds as “legitimate” pollinators of this species. According to GOBATTO-RODRIGUES & STORT 1992 and CAMARGO & al. 1984, bees such as Xylocopa ssp., Bombus ssp., Oxaea flavescens, and Scaptotrigona postica also intensively visit *Pyrostegia* flowers, however, these bees perform nectar robbery, often piercing or gnawing the corolla tube basis. This behavioral pattern obviously causes depletion of the nectar supply. Nevertheless, some evidence shows that nectar robbing by bees from orange flowers can affect the activities of legitimate pollinators, because the decreased nectar quantities in the individual flower force the hummingbirds to move from flower to flower more frequently so that pollen flow might be intensified (CAMARGO & al. 1994).
It was not possible to observe legitimate and illegitimate flower visiting at yellow flower forms of *Pyrostegia*, because the whole natural vegetation of the area was converted for agricultural purposes before the present study was completed. Therefore, it is not known if the differences between orange and yellow flower forms indeed alter the foraging behavior of legitimate and illegitimate flower visitors, in particular with regard to visiting frequency or competition for nectar resources.

Since apparently nobody else before has noted yellow-flowered individuals of *P. venusta*, it might be speculated that they are the result of a spontaneous local mutation, with a modification in flower morphology, petal pigments, and nectar composition.

In general, yellow-colored flowers are usually more attractive to bees, and orange-colored ones can be more attractive to hummingbirds (Harborne 1989), but there are also many exceptions. The flowers of both color morphs contain carotenoids and flavonoids. Many flavonoids together with flavonols serve primarily as ultraviolet-absorbing pigments, supplementing other major groups of floral pigments, the carotenoids and anthocyanins, which account for most of the visible color of flowers (Geissman 1963, Thompson & al. 1972). It is well known that bees can discern differences in UV absorbing pigments that are of widespread occurrence in flowers. Although UV patterns are not frequently associated with ornithophily, they might play a role in this syndrome (see Jacobs 1992, Biedinger & Barthlott 1993, Burr & al. 1995). Indeed, both color morphs fit the syndrome of hummingbird-pollinated species morphologically. On the other hand, and according to Camargo & al. 1984, and Gobatto & Stort 1992, the orange flowers are in addition commonly visited by many bee species, which are nectar robbers; this might be in part a response to a UV absorbing pattern of these flowers. These authors also observed that the bee visitors usually do not contact the reproductive organs but pierce corollas or use the holes made at the base of the corolla tubes.

Without data it would be pure speculation to assume that in a mixed population with orange and yellow flowered individuals, hummingbirds would preferentially visit orange flowers, and bees more frequently the yellow ones. On the other hand, the positive caloric budget of orange flowers as compared to yellow ones, together with the capacity of hummingbirds to learn flower colors should elicit the visits of hummingbirds preferentially to orange flowers, which they might associate with a higher caloric reward. To satisfy the daily energy requirement of a hummingbird, which is 6 to 12 Kcal (Stiles & Wolf 1971, Wolf & Hainsworth 1971), only on *P. venusta*, a hummingbird would need to visit an average of 87 orange flowers (ingesting a 8.8 ml nectar volume) but as many as 141 yellow flowers (ingesting a 11.2 ml nectar volume). If we would assume further that flower tube piercing and nectar robbing by bees
might be even stronger in yellow flowers than in orange ones, the yellow-flowered individuals might become relatively unattractive for hummingbirds within a population where both flower color morphs are present. As a consequence, the reproductive success of the yellow-colored form should be lower than that of the original orange-colored one, which would explain the low number of individuals as well as the small size of the yellow population and may even lead to its natural disappearance after some time.

The sucrose dominance in orange and yellow flowers of *Pyrostegia* is a further characteristic of the hummingbird syndrome (see Stiles 1976, Hainsworth & Wolf 1978, Baker & Baker 1979, 1983). Sugar concentration detected during this study is lower than values found by Galetto & al. 1994 in Argentina, and also lower than data given by Gottsberger & al. 1984 from Botucatu, state of São Paulo. This might be an indication to be confirmed, that different populations within the distribution range of the species behave differently with respect to nectar production and sugar content. This also may concern amino acid concentration (compare data of Gottsberger & al. 1984 with those of Galetto & al. 1994, and the present study).

Differences in amino acid concentration in orange and yellow flowers certainly have no influence on pollination by hummingbirds, even when piercing of flowers by bees may drastically increase amino acid concentration (see Gottsberger & al. 1984). Hummingbirds, which catch insects as food, certainly do not rely on the amounts of amino acids occurring in nectar (e.g. Baker & Baker 1983).

In conclusion, *Pyrostegia venusta* is a self-compatible and facultatively xenogamous (Gobatto-Rodrigues & Stort 1992) neotropical vine, which is legitimately visited and pollinated by different hummingbirds throughout its geographical range, and illegitimately visited and nectar robbed by several flower-piercing bee species. The uncommon yellow-flowered form appears to be a multigenic mutant, which perhaps is accessible only to a reduced number of hummingbird species because of a straighter floral tube as compared with the orange-flowered form. Its supposedly lesser attractivity to hummingbirds due to its lower caloric reward and its reduced reproductive capacity because of its lower pollen/ovule ratio as compared with the orange form might tentatively explain its rare and local occurrence.

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Recensiones
