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# Relationship between peroxidase activity and flower localization along *Vanilla planifolia* vines

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## Abstract

Decapitated pending vines are the most responsive parts of vanilla plants. Inflorescence localization along training vines showed a decreasing gradient from the decapitated end to the base in sun locality and was irregular and showed opposite gradient in shade locality. Soluble and ionic peroxidase activities, determined in the leaves and in the internodes, showed a marked peak during the end of June and a gradient along training vines which was inverse to the gradient of inflorescence localization and opposite between sun or shade conditions, supporting the utility of peroxidases as spatial and temporal biochemical markers of flowering processes.

*Key words*: decapitation, inflorescence, ontogeny, shade and sun conditions.

## Introduction

The orchid, *Vanilla planifolia* Andrews, is mainly cultivated in the countries of the Indian Ocean (Indonesia, Madagascar, Reunion Island and Comores) for the production of vanilla pods and vanillin aroma. Vanilla flower and fruit productions in Reunion Island showed large fluctuations in the past eight years while very few is known so far on flowering control in this species. In a former paper (Fouché and Coumans 1995), a proposed period for flowering induction in June and solar irradiance as a parameter involved in the floribundity were deducted from the relationship between monthly insolation and vanilla pod production and the detrimental effect of shade during this month.

Several studies have been devoted to flower induction in *Orchidaceae*. In most orchids it is induced by cool temperatures (Goh 1979), photoperiod (Bose and Mukhopadhyay 1977, Goh *et al.* 1982), irradiance (Tran Thanh Van 1974)

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or drought (Goh *et al.* 1982). Growth regulators have also been applied successfully on some orchid genera (Brewer *et al.* 1969).

Flower induction in vanilla usually starts during June in Reunion Island and it is dependent on irradiance as suggested by the good correlation between vanilla pod production and insolation during the month of June (Fouché and Coumans 1995). Peroxidase activity have been used as biochemical marker to characterize the induction phase of the flowering process (Gaspar *et al.* 1985, Greppin 1986, Bouazza *et al.* 1993).

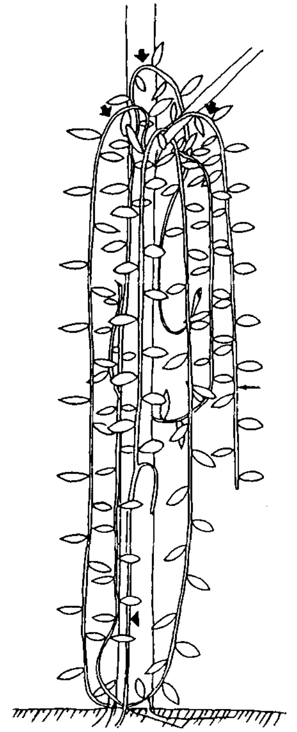

Fig. 1. Schematic drawing of a freshly pruned *Vanilla* plant conducted up and down on its support (*arrowhead* - starting cutting; *small arrow* - pendant decapitated end; *large arrows* - curvatures).

To understand how and why a vanilla plant flowers, it is important to know how it grows in open fields or underwood. The architecture of the vanilla plant with special attention to flowering was studied (Fouché 1992). In that work, inflorescences have been localized on training vines as flowering improvement was obtained by the creation of training vines with decapitated pendent tips (Leakey 1970, Fouché 1992). Furthermore, using the Markov theory (Ruegg 1989), the floral or non-floral states of the nodes have been analysed as a stochastic process of apparent chaos ordering. Statistical modelization has already been used to mimic plant development such as growth and architecture of *Coffea robusta* plants (De Reffye 1982).

The aim of the present work was to test the use of peroxidases as a biochemical marker of flower induction and of the inflorescence distribution along the vines.

## Materials and methods

**Material:** *Vanilla planifolia* Andrews (syn. *V. fragans*), an orchid introduced in Reunion Island between 1819 and 1821, is propagated vegetatively. Plants from two locations were used in experiments: (*1*) a vanilla experimental lot (Colosse field) where vanilla was planted on *Draceana reflexa* as support and with sugarcane intercalation, and (*2*) a private plantation growing under sun conditions on *D. reflexa* and with sugarcane or under shade conditions in underwood with large leafy trees.

The vines were conducted up and down on the support on a 1.80 height. Pruning was done at the end of May to create training vines of approximately 20 nodes long before the first curvature (Fig. 1).

Leaves and internodes from basal (numbered 18, 19 and 20 from the decapitated end), medium (9, 10 and 11) and apical (1, 2 and 3) regions for each vine were sampled systematically from June to August. For each measurement, 3 nodes per vine were used; each experiment was repeated ten times.

**Peroxidase activity:** 3 g of material stored at -20 °C were ground for 10 min in the cold in 0.01 M phosphate buffer, pH 7.0, in the presence of 1 g of polyvinyl-pyrrolidone (PVP-10, *Sigma*). After centrifugation at 10 000 *g* at 1 °C, the supernatant constituted the soluble fraction. The pellet was reextracted with the same buffer in the presence of 1 M NaCl. Centrifugation at 10 000 *g* gave as supernatant the so-called ionic fraction. By this procedure, 85 % of the extractable soluble + ionic peroxidases were obtained.

Peroxidase activity was determined spectrophotometrically using guaiacol according to Darimont and Gaspar (1972). Proteins were assayed by the biuret method (Gurd 1970).

## Results

The inflorescences of *Vanilla* plants can develop anywhere along the vines. To undertake biochemical studies with potential floral nodes, it was important first to localize more precisely where flowers would appear.

Under sun conditions, the maximum of inflorescences was found on the first node beside the cut end (35 %) and the frequency of floral nodes progressively decreased to the base of the vine (Fig. 2). The mean for this experiment was 3.26 inflorescences per training vine. When the vine was not decapitated, inflorescences never developed at a distance of less than 20 nodes from the apex.

On vines grown in shade conditions was a substantial decrease in flowering and most of the inflorescences were scattered along the vines away from the decapitated ends with an estimated increase from the top to the base of the vines.

Standard deviation in soluble and ionic specific peroxidase activities was important (from 18.9 to 61.0 % of the value with a mean of 32.4 %) and was partly due to the heterogeneity of vanilla plants in plantation, even after selecting relatively uniform material. Nevertheless, there was a significant increase in activity between

12 June and 9 July in all conditions. It was very important in shade conditions where it could reach 15 times or more the initial level, while it was five times smaller in sun conditions (Fig. 3).

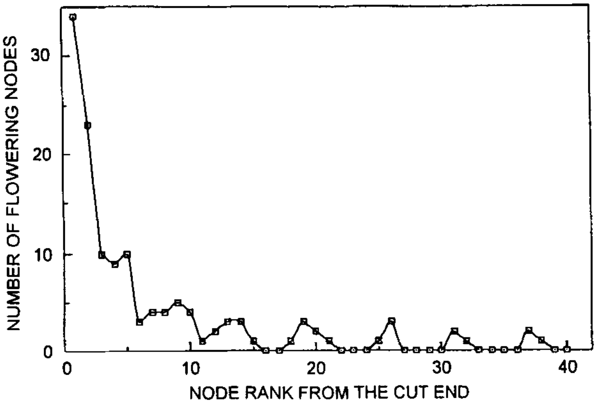

Fig. 2. Frequency of inflorescence localization along pending decapitated *Vanilla* vines.

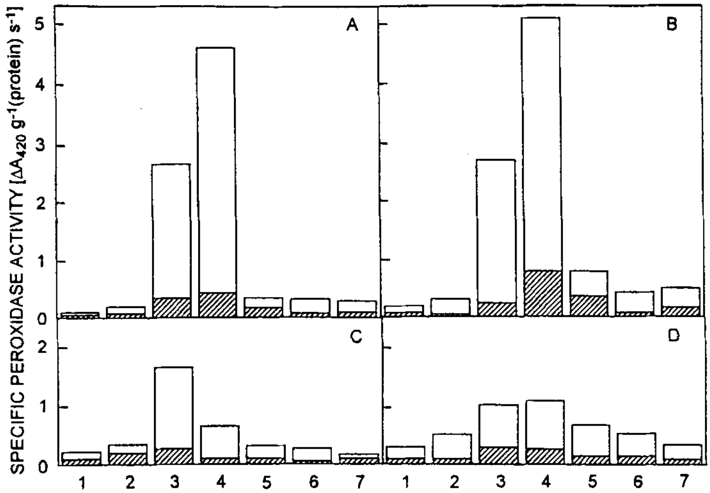

Fig. 3. Specific soluble (*hatched columns*) and ionic (*empty columns*) peroxidase activity in leaves (*A* and *C*) and internodes (*B* and *D*) under shade (*A* and *B*) and sun (*C* and *D*) conditions.

This peak of activity was present in basal, medium and apical parts of the vine but amplitude followed a gradient. Basal part showed the highest activity in sun conditions and the activity never reached 0.16 activity units. On the contrary, under shade conditions, the highest activity was located in the apical part with a total soluble plus ionic peroxidase activity of nearly 0.60 units (Fig. 4).

## Discussion

The month of June has been shown to be critical for the induction of *Vanilla* inflorescences (Fouché and Coumans 1995). This is confirmed by the fact that important cultural treatments, *i.e.* apex decapitation, vine curling and sugarcane harvesting have to be done at end of May or beginning of June for maximum efficiency.

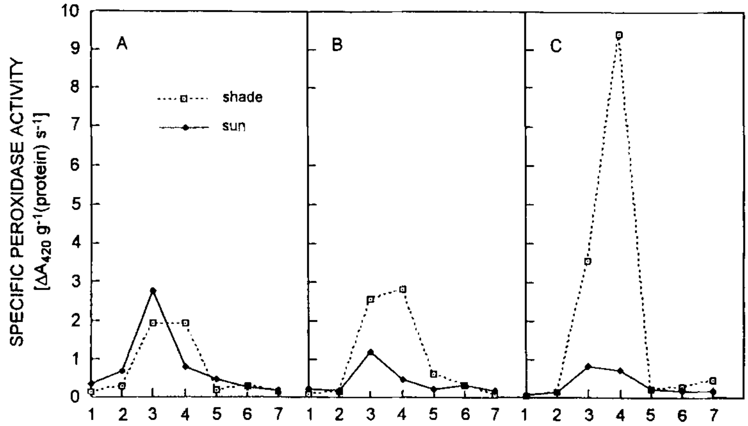

Fig. 4. Specific soluble + ionic peroxidase activity. Comparison in sun and shade conditions among base (*A*), medium (*B*) and apical part (*C*) of the decapitated training vines. Sampling dates: 5 June (*1*), 12 June (*2*), 25 June (*3*), 2 July (*4*), 9 July (*5*), 23 July (6) and 15 August (*7*).

The presence of a peak of peroxidase activity during this period both in the sun and shade conditions, is a sign that an unknown signal, responsible for peroxidase activity increase, has been received by the whole plant during this period. The modulation of peroxidase activity has already been proposed as a quick indicator of the start of floral induction (Greppin 1986). It is still hypothetical that this signal would be directly implicated in flowering induction in vanilla. As possible environmental factors involved can be mentioned the photoperiod (21 June as the shortest day) and the temperature (the lowest nocturnal temperatures in June). An effect of irradiance could be hypothesized through an increase in photosynthates as saccharose has already been related with flowering induction in *Sinapis alba* (Lejeune *et al.* 1993).

The activities of peroxidases could be good biochemical markers of flowering. The activity gradient along the vines is opposite in sun and shade vines similarly as the gradient of inflorescence localization.

Flowering induction was also related to IAA-oxidase activity (Gaspar *et al.* 1975). The implication of IAA degradation by boron applications (Dugger 1985) in June could also be considered (Fouché 1992).

The role of auxin in flowering has been ascertained by correlation between inductive treatment and fluctuation of auxin level. It has been shown, for example, that flower induction for *Chenopodium rubrum*, a short day plant, was accompanied by an increase in peroxidase and IAA oxidase activities (Krekule and Macháčková

1986) and by low IAA content (Pavlová and Krekule 1984) even if *in vivo* IAA metabolism studies did not clearly confirm the primary role of IAA-oxidases in free IAA level regulation (Krekule and Macháčková 1986 ). In *Sinapis alba*, the flowering induction by long days resulted in a decrease of the auxin level in the apical buds (Sotta *et al.* 1992). Jacobs (1985) has proposed that auxin may function as a general inhibitor for transition to reproductive phase.

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